

**EFFECTS AND CONTROL OF BARNACLE  
INFESTATION ON MANGROVE SEEDLINGS IN A  
MANGROVE REHABILITATION SITE**

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**FACULTY OF SCIENCE  
UNIVERSITY OF MALAYA  
KUALA LUMPUR  
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**TAN YEE SIANG**

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## ABSTRACT

A study on the development, effects and control of biofouling on replanted mangrove seedlings (40-50cm height) was carried out, from April 2008 to July 2010, at Kampung Sungai Haji Dorani and Kampung Sungai Limau, Selangor, Malaysia. The dominant biofoulers were barnacles (*Amphibalanus amphitrite*), which were the main causal organism of mangrove seedling damage or death. The high density (>100 no./ 10 cm section of plant stem) and cover (>30 %) of barnacles appeared to obstruct development of new leaves, shoots and roots, as well as imposing weight loads as high as six times the weight of the seedlings. The 10-20cm section of the stem above ground had the highest number of barnacles (mean =  $125 \pm 106$ ;  $p < 0.05$ ). However, growth and survival of seedlings with low percentage cover (<30%) of barnacles and other fouling organisms were not affected over the 20 weeks of study; these were seedlings planted on higher ground (2.0-2.5m above chart datum). Histological study showed that the percentage thickness of the palisade layer of fouled leaves was significantly ( $p < 0.05$ ) lower compared to non-fouled leaves, but there was no significant difference ( $p > 0.05$ ) in the number of abaxial hairs. The mean abundance of barnacles attached on the seedlings planted using the 'conventional' stake method (wooden stake tied to the seedling) (3710 no./plant) was significantly higher compared to seedlings planted inside PVC piping or encasements (25.6 no./plant) after 10 weeks. Another study conducted using three planting methods showed that barnacle abundance in the elevated planting method (seedlings raised up by 15cm above ground by planting them inside a cylindrical 'net pot') (1.5 no./plant) was significantly ( $p < 0.05$ ) lower compared to that of seedlings planted using the short stake method (a short wooden stake of 10 cm tied to the base of the plant) (5.5 no./plant) and conventional method (21.3 no./plant). Results support the contention that reducing the inundation time of mangrove seedlings by the flood tide, either by raising their height above ground, planting them on higher ground,

or using taller seedlings will reduce or ameliorate the effects of barnacle infestation. In the chemical control study, weekly topical applications of Clorox, Ivermectin, Neguvon and tea seed extract significantly ( $p < 0.001$ ) reduced the abundance of barnacle infestation as compared to the control (distilled water). Ivermectin and Clorox however caused negative side effects to crabs, but not to gastropods and fishes. Other factors controlling barnacle infestations and recommendations on their control are discussed.

## ABSTRAK

Masalah *biofouling* (terutamanya teritip) telah dikenalpasti sebagai salah satu punca yang menyebabkan kematian anak pokok bakau. Objektif kajian ini adalah untuk menentukan spesies, tumbesaran, kesan dan kawalan *biofouling* pada anak pokok bakau demi membantu usaha kerajaan dalam program penanaman anak pokok bakau. Kajian ini dijalankan dari April 2008 hingga Julai 2010 di Sungai Haji Dorani dan Sungai Limau, Selangor, Malaysia. Organisma *biofouling* yang dominan dan menyebabkan kerosakan dan kematian anak pokok bakau adalah teritip (*Amphibalanus amphitrite*). Anak pokok yang ditanam dengan kaedah konvensional pada 1.5 meter di atas Carta Datum tercatat purata 3710 teritip/pokok pada minggu ke-10. Densiti ( $>100$  bil./10 cm bahagian batang pokok) dan liputan (*cover*) ( $>30\%$  permukaan pokok) teritip yang tinggi menghalang pertumbuhan daun baru, tunas dan akar dan mengenakan beban berat enam kali lebih daripada berat anak pokok. Muatan ini akan menyebabkan batang pokok menjadi bengkok. Bilangan teritip yang paling tinggi (mean =  $125 \pm 106$ ;  $p < 0.05$ ) didapati pada bahagian batang 10-20 cm atas tanah. Walaupun demikian, pertumbuhan dan kemandirian anak pokok (*Rhizophora apiculata*, *Rhizophora mucronata* dan *Avicennia marina*) yang mempunyai liputan teritip yang rendah ( $<30\%$  / pokok) tidak terjejas, ini disebabkan anak pokok bakau ditanam lebih tinggi (Carta Datum 2 m-2.5 m). Keputusan kajian histologi pada permukaan daun yang ada teritip menunjukkan peratus ketebalan lapisan *palisade* yang lebih tinggi berbanding permukaan daun yang tiada teritip, tetapi bilangan *abaxial hairs* didapati tidak ada perbezaan yang signifikan ( $p > 0.05$ ). Kajian untuk mengurangkan bilangan teritip pada anak pokok bakau terbahagi kepada kaedah fizikal dan kaedah kimia. Kaedah fizikal menunjukkan keputusan bahawa purata bilangan teritip pada kaedah menanam *elevated* (1.5 bil/pokok) adalah kurang secara signifikan ( $p < 0.05$ ) berbanding dengan kaedah menanam *short stake* (5.5 bil./pokok) dan kaedah menanam konvensional (21.3 bil./pokok). Keputusan ini menyokong pendapat

bahawa jikalau masa rendaman anak pokok (oleh air pasang) dapat dikurangkan dengan cara menanam anak pokok lebih tinggi atas tanah ataupun menanam pokok yang tinggi/besar, maka infestasi/kesan teritip dapat dikurangkan. Bahan kimia yang digunakan untuk mengawal teritip adalah seperti *Clorox*, *Ivermectin*, *Neguvon* dan ekstrak *tea seed*. Bahan kimia yang disapukan pada permukaan anak pokok setiap minggu. Keputusan menunjukkan pokok yang dirawat dengan *Clorox* (52.2 bil./pokok), *Ivermectin* (43.1 bil./pokok), *Neguvon* (55.2 bil./pokok) dan ekstrak *tea seed* (53.2 bil./pokok) dapat mengurangkan min bilangan teritip secara signifikan ( $p < 0.001$ ) berbanding dengan kawalan (air suling) (95.3 bil/pokok). Walaubagaimanapun, keputusan tentang kesan bahan kimia terhadap hidupan di kawasan kajian menunjukkan bahawa *Ivermectin* dan *Clorox* akan mengurangkan bilangan ketam, tetapi tidak terhadap haiwan ikan belacak dan gastropoda. Faktor-faktor lain tentang pengawalan *biofouling* di Haji Doraini juga dibincangkan.

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# CHAPTER 1: INTRODUCTION

## 1.1 An overview of biofouling

In general, biofouling is regarded as the colonization of hard surfaces by living organisms on wetted structures (Railkin, 2004). Fouling refers directly to the accumulation of unwanted matter on the surface of an object. By using the prefix *bio*, it then refers to accumulation of a living community at the interface (Simone Dürr & Thomason, 2010). Kingsbury (1981) defined marine biofouling as a collective term for organisms growing on artificial structures placed in marine and estuarine environments. Flemming (2002) defined biofouling as also the unwanted deposition and growth of biofilms. Biofouling occurs as a result of settlement and growth of sedentary and semi-sedentary organisms on artificial structures placed in water (Venugopalan & Wagh 1990). Baretta-Bekker *et al.*, (1992) indicated biological fouling or biofouling as growth of sessile algae and animals, especially on a ship's bottom or other artificial underwater structures.

Around the world, there are more than 2000 species of biofouling organisms including 50 species of bacteria, 110 species of diatoms, 450 species of algae and 1900 species of other animals (Hutchins, 1952 cited in Cook, 2001). Anderson and Hunter (2000) had reported that the number of biofouling organisms have increased to more than 4000 species worldwide. In Malaysia, Cheah and Chua (1979) recorded more than 34 species of fouling organisms encrusting floating net-cages which were dominated by oysters, algae tunicates and mussels.

Biofouling can be classified into microbiofouling and macrobiofouling (Delauney *et al.*, 2010). The biofouling process begins with adsorption of complex

molecules onto exposed surface that attract bacteria onto it, followed by diatoms, and together with particulate matter, forms the biofilm. This is often collectively called microbiofouling. Microbiofoulers are mainly represented by sessile bacteria, diatoms, microscopic fungi, heterotrophic flagellates, sarcodines and sessile ciliates (Abarzua & Jakubowski, 1995). It is believed that this biofilm, or its emanated chemo-substances, is responsible for the initiation of the fouling succession. What follows is termed macrobiofouling which begins with the settlement of larvae of sessile or attached forms. Macrofouling organisms include sponges, hydroids, corals, sessile polychaetes, barnacles, mussels, bryozoans, sea cucumbers, ascidians and macroalgae (Madin *et al.*, 2009). Macrobiofouling organisms will exhibit gregariousness and compete for the space, food, sun light and other resources in the habitat (Raikina, 2004; Ferson 1950). The sessile macroorganisms inhabiting hard surfaces, in turn, serve as substrates for new sessile organisms of the second, third, and higher orders involved in the process of successive colonization of the surface (Seravini *et al.*, 1985). Thus, these communities acquire a characteristic multilayered vertical structure (Partal, 1980; 2003).

In microbiofouling, the dispersal forms are their vegetative (and sexual) cells, and also spores and cysts, which may be carried by water and air current to long distances, resulting in their ubiquitous distribution. The dispersal forms of macroalgae are motile or immotile spores, whereas those of invertebrates and ascidians are motile larvae. In their distribution by currents, an important role is also played by their own motility and selectivity in the choice of substrates (Raikina, 2004).

Higher abundance of biofouling organisms in the coastal zone and on the shelf, as compared to the deeper areas of the ocean may be due to the considerable number of available substrates for settlement of the propagules (larva and spores) (Kusakin &

Lukin, 1995). The best trophic, temperature and photic conditions in this shallow part of the ocean contribute to the growth and reproduction of organisms (Valiela, 1984; 1985). It seems to be also important that the coastal currents retain some of the dispersal forms, sometimes a considerable number, in the shelf zone without carrying them into the open sea (Martin & Foster, 1986).

Ship and flotsam have important role in the random dispersion of different species of marine animals and plants to great distances, even between continents (Scheltema, 1971; Zvyagintsev, 2000). As a result of larval drift, the transport of the foulers by ships and flotsam, a number of invertebrates and macroalgae are carried to geographical zones, regions, and biotopes new to them. This results in the extension of ranges of these species, in their biological progress, and, in some cases, they replace native species. The problem of invasion by species and disturbance of marine ecosystems is one of the ecological problems of the twentieth century (Carlton *et al.*, 1990; Zvyagintsev, 2000)

### **1.1.1 The effect of biofouling**

Biofouling poses significant problems particularly to the shipping industry. It is estimated that biofouling incurs yearly losses of over US\$6.5 billion to the global shipping industry mainly from higher fuel consumption and regular maintenance (Callow & Callow, 2002). Biofouling growth on the hull of a ship would greatly increase friction between the ship's surface and the surrounding water. To overcome this resistance, extra fuel is needed and thus, increasing the cost of operation (Redfield & Ketchum, 1952; Morton & Gaylarde, 2001). Biofouling organisms will caused the smooth surface becomes rough and sometimes even knobby (Tarasov, 1961). By

increasing the hull roughness by only 0.025mm raises its friction resistance by 2.5% and result in extra fuel consumption (Gurevich *et al.*, 1989). The dense layer of macrofouling, such as bryozoans, on the ship may screen the release of toxic substances from antifouling coating (Raikin, 2004). Biofouling damages pier pilings and fish net cages causing economic losses (Chan & Lee, 2007; Madin, 2010; Railkin, 2004). Intensive fouling reduces pipelines carrying capacity, hampering their operation and sometimes (in case of blockages) leading to the breakdown of the units and mechanisms cooled by water (Adamson *et al.*, 1984). A frequency cause of ship wreckage is engine failure owing to the heavy biofouling of fuel lines (Bowes, 1987). Biofoulers may totally block the piping, disrupting the fuel supply and stalling the engine. Biofouling also accelerates corrosion of the metal walls of heat exchangers (Adamson *et al.*, 1984). The layer of micro- and macrofoulers serves as a buffer between service water and water pumped in from the sea, which results in energy losses and premature wear of different machines and mechanisms (Adamson *et al.*, 1984). Biofouling of net-cages will significantly reduce the size of net mesh, impedes water flow through the net (Madin *et al.*, 2009; 2010). Marine foulers are capable of destroying objects made of varieties of materials. Some of them make tunnels in wood, destroy concrete, and damage the shells of commercially important mollusks (Raikin, 2004). In Putatan and Lahad Datu (Sabah, Malaysia), the high mortality rate of replanted mangrove seedlings is suspected to be caused by heavy fouling of barnacles (Anonymous, 2007).

However, in several aspects, biofouling have brought benefit to human and environment. For example, oyster and mussel beds work as real biofilters, passing vast volumes of water through themselves, extracting pollutant and pathogenic microorganisms and thereby purifying and clearing water (Alexandrov *et al.*, 2002). The use of filter feeding fouling organisms can minimize the impacts of pollution in

aquaculture farms (Coasta-Pierce & Bridger, 2002). Communities foulers can attract other organisms and the productivity of plankton is high. For example in the zone of the blue mussel mariculture, the biomass of algal plankton is 10 times as high as that in the adjacent water areas without mussels (Galkina *et al.*, 1982) which attracts large aggregation of fish (Zenkevich, 1977; Sorokin, 1993; Gromov *et al.*, 1996).

### **1.1.2 An over view of anti-fouling**

Anti-fouling is the process of removing accumulated, or preventing the accumulation of biofoulers (Madin, 2010). Examples of biofouling protection systems found in the market include purely mechanical devices such as wipers or scrapers, “uncontrolled” biocide generation systems based on the copper corrosion mechanism or tributyltin (TBT) biocide leaching, and “controlled” biocide generation systems based on a localized seawater electro-chlorination system or an automatic acid dispensing device (Bleile & Rodgers, 1989; Alberte *et al.*, 1992; Yebra *et al.*, 2004; Delauney *et al.*, 2010).

The simplest method of physical protection against biofouling is to create a mechanical barrier to shield the surface from settling propagules (Rasmussen, 1969). Another example of physical protection is through flaking of paint, in which case the paint layer would peel off the surface together with the organisms attached to them. Organotin-containing self-polishing copolymer (SPC) coatings, originally designed by the International Paint Marine Coatings (United Kingdom), have been in use in many countries since the date 1970s (Railkin, 2004). The antifouling properties of SPC result from the joint effect of chemical and physical factors. As a result of the hydrolysis of the covalent bond between the biocide (for example TBT) and a polymeric matrix, the

former is released into the boundary layer too lethal for settling propagules. Due to the self polishing properties, the SPC coating can last for 5 years and longer (Clare, 1996; Frost *et al.*, 1999). The other type of self-polishing coating, ABC (ablative coatings) class, immersed in water, the organosilicon polymer dissolves slowly, releasing biocide, and the biofouling flakes off (Yuki & Tsuboi, 1991). Ultrasonic method of protecting vessels (Fischer *et al.* 1984, Shadrina 1995) and objects of mariculture (Lin *et al.*, 1988) from biofouling are being developed. Ultrasound is based on the mechanical destruction of firmly attached organisms by acoustic vibrations at frequencies ranging approximately from 20-200 kHz and pulse radiation power up to 1kW. However, this method has to be used in combination with antifouling paints (Shcherbakov *et al.*, 1972) or with the electrolysis of sea water (Edel'kin *et al.*, 1989) for more effective protection against marine biofouling. Radioactive isotopes for example technetium-99 is effective protecting against biofouling (Makarova, 1990). However it is unlikely to become widely used because of its high health and environment hazards. There are other approaches to physical protection against biofouling such as high temperature, magnetic and electric field, current, hydrodynamic force and even blast waves (Fischer *et al.*, 1984; Gurevich *et al.*, 1989). Other ways to control biofouling are the use of propagule-free filter water in cooling systems; by creating a mechanical barrier to fence off the propagules; by maintaining a liquid layer over the protected surface to prevent attachment or by removing the foulers that settled on and attached to the surface (Raikin, 2004).

The methods of chemical protection include antifouling coatings, chlorination, ozonation, treatment with copper sulphate, anodic protection and plating of the surface (Fischer *et al.*, 1984). The chemical protection is carried out mainly with the use of copper, zinc, and lead oxidesorganotin compounds; chlorine; and ozone (Raikin, 2004;

Yebra *et al.*, 2004). Chemical methods are effective to prevent biofoulers; however, heavy metals, arsenic and free chlorine constitute major contaminants to the aquatic environment. TBT and TBTO (tri-n-butyltin oxide) have been found to be toxic to many marine organisms (Ellis, 1991). The hazards of heavy metals include high toxicity which circulate in food chains, and may be capable of accumulating in an organism. It is necessary to note that the use of triorganotin in ship paints was supposedly banned as of 2003, but it unfortunately continues to be used in third world countries (Rittschof, 2001). Various marine natural product antifoulants, for example, renilla-foulins (in seapens), halogenated furanones (in red algae), pukalides (in corals) and diterpenes (in sponges) have been tested for potential industrial applications (Fusetani, 2004).

## **1.2 An over view of barnacles**

### **1.2.1 History**

Charles Darwin published two monographs on the living species (Darwin, 1851a; 1854a) and two on the fossil species (Darwin, 1851b; 1854b) of barnacles that remain the foundation of cirripede biology today. The taxonomy and distribution of the group is now well established on a worldwide basis, with key contributions from Annandala, Aurivillius, Broch, Gruvel, Hoek, Krüger and Nilsson-Cantell in the year of 1930, Hiro (later Utinomi) in the middle years of this century and Foster, Newman, Ross and Zevina in the more recent times (Anderson, 1994). The understanding of barnacle fossil history has been further advanced by Withers, and later by Zullo and by Buckeridge. Barnes, Crisp, Southward, Connell and Underwood are among those who have studied barnacles' productivity. Newman, Zullo and Withers (1969) had placed considerable emphasis on fossil history and phylogenic evolution, and also reviewed advances in barnacle morphology and ontogeny. Southward (1987) had treated many aspects of the

biology of the group including comprehensive bibliographies. Klepal (1987; 1990), Barnes (1989) and Walker (1992) had also reviewed the biology of barnacles.

### **1.2.2 Morphology and feeding mode of barnacles.**

By common consent, due to barnacles' worldwide occurrence in costal and shallow water, species of *Lepas* have been used to typify stalked cirripedes and species of *Balanus* the non-stalked forms (Anderson, 1994). The titles of Darwin's two monographs, one on the Lepadidae, the other on the Balanidae, reflect *Lepas* and *Balanus* as focal barnacles 'types'. The major anatomical feature of *Lepas anatifera* Linnaeus and *Balanus trigonus* Darwin are embodied in the definitions attached to the major steps in the hierarchical classification of the Cirripedia (Newman, 1987), as follows:

Phylum Crustacea Pennat, 1777

Superclass Maxillopoda Dahl, 1956

Class Thecostraca Gruvel, 1905

Subclass Cirripedia Burmeister, 1834

Superorder Thoracica Darwin, 1854a

Order Pedunculata Lamarck, 1818

*Lepas anatifera* Linnaeus, 1758

Order Sessilia Lamarck, 1818

*Balanus trigonus* Darwin, 1854a

*Balanus* is a low conical barnacle with the aperture in an apical position.

*Balanus* sits on a broad basis, firmly cemented to the substratum. The basis of *Balanus*



is strengthened by a calcified plate. The plates of *Balanus* are clearly arranged as a fixed, conical wall and an apical, movable operculum. The operculum comprises left and right opercular valves, able to move apart laterally and to close tightly during the animal's various activities. Internally, the operculum, wall and basis of *Balanus* are lined by a mantle, enclosing a mantle cavity containing the body of the animal. Only the last three pairs (cirri IV-VI) retain long rami and contribute to the extension of a captorial cirral fan. Cirri III, II and I are modified as maxillipedes. The maxillipedes interact together in a complex manner during the rhythmic beating of the cirral fan to transfer captured food from the fan to the mouthparts. Barnacles do not absorb nutrients from objects that it attaches on. Barnacles are filter feeders. When covered by sea water, barnacles will open their operculum (scutum and tergum) and extend their feathery appendages (cirri) to filter microscopic and planktonic foods from the water and then transfer them to their mouth (Anderson, 1994).

### **1.2.3 Life cycle of barnacles**

Barnacles are one of the main fouling organisms of ships and any hard structures in the sea (Foster, 1967). There are more than 1000 known species of barnacles (Chan & Lee 2007). Acorn barnacles are commonly found in the estuarine and intertidal zone attaching themselves on to any hard objects submerged in sea water such as a ship hulls, water cooling intake pipes in power plants and pillars of ports, sea walls, shore line protective structures and organic surface of crabs, molluscs, roots and the lower region of mangrove trees, shell of sea turtles and even on the body surface of whales (Raikin, 2004; Grunbaum, 2010; Madin, 2010).

Most barnacles are hermaphrodites, where an individual possesses both male and female sex organs. However, it has been shown that a hermaphroditic barnacle prefers to fertilize neighboring barnacles. Of all known animals, the barnacle has the longest penis in relation to its body length allowing it to increase its range and possibility of finding and mating with a partner (Anderson, 1994; Chan & Lee 2007).

The life cycle of barnacles begins with a planktonic larval stage which is then followed by the sessile adult stage. After mating, a brood of fertilized eggs will develop into naupliar larvae and these larvae are released into the water column. Most of the nauplii will molt six times to metamorphose into the cypris. A cypris has more appendages than the nauplius but it does not feed. When it finds a suitable place to settle down, the gland in its antennae will secrete a glue to attach itself permanently to a substrate and undergo metamorphosis to become a juvenile barnacle. Barnacle cypris can sense chemical cues released by the same species and tend to settle near its own species (Anderson, 1994; Chan & Lee, 2007; Tan, 2008).

### **1.3 An overview of mangrove**

Mangrove forest, or mangal, are found between mudflat and riverbanks in tropical and subtropical regions in between latitudes 25°N and 30°S (Macnae, 1969). It can be found at Southeast Asia, the Sundarbans of Bangladesh and India, and along the Orinoco River of Venezuela (Ellison, 2000). Globally, mangroves occur in 114 countries and territories and the total global area has been estimated at 181,000 km<sup>2</sup> (Spalding, 2004). True mangroves are species which are adapted to the mangrove environment and do not extend into other coastal plant communities. True mangroves

consist of a core group of some 30-40 species (Spalding, 2004). True mangroves have a number of adaptations that help them to survive in the intertidal zone. These adaptations include possession and ability of mechanical fixation in loose soil, breathing roots and air exchange devices, specialized dispersal mechanisms, and specialized mechanisms for dealing with excess salt concentration (Spalding, 2004). Mangroves are the only true viviparous plants which mean that the seed remains attached to the parent plant and germinates into a protruding embryo (propagule) before falling from the tree (Rey, 1999).

### **1.3.1 The importance of mangroves**

Mangroves play an important role in soil sedimentation and stabilization. Mangrove trees are able to reduce the impacts of strong waves and tidal currents thereby protecting the shoreline from erosion (Imbert *et al.*, 2000). For example, when a typhoon hit the Zhujiang Estuary, it over- flowed and submerged 2129 places caused a 47 km long seawall to collapse, damaged 32 bridges and drowned 1533.3 km<sup>2</sup> of farm land and fish ponds. However, the seawall at the east coast was safe as it was protected by tall mangrove trees, reeds and (Zhao *et al.*, 1994). Some countries for example Bangladesh have established mangrove plantations to stabilise sediments and to reduce the impact of storm surges (Saenger & Siddiqi, 1993).

Mangrove trees provide both food and habitat for the diverse communities of fauna, for examples birds, snakes, mollusks, fishes, prawns, crabs, barnacles, insects and zooplankton (Sasekumar *et al.*, 1994; Chong, 2007; Chew, 2010). Mangroves also serve as nursery grounds for juvenile prawns and fish (Blaber *et al.*, 2000).

Mangroves have the ability to improve water quality by lowering the nitrite and phosphate concentrations and absorb heavy metal pollutants from the water (Ewel, 1998). In economic terms, humans harvest wood from mangroves to make charcoal, scaffoldings, boats and furniture. Fauna like crabs, mollusks, fish and prawns are sources of food that can be obtained from mangrove forest. A positive correlation between near shore yields of fish or shrimp and mangrove area has been documented in the Indonesia (Martosubroto & Naamin, 1977) and Malaysia (Macnae, 1974). More recently, mangrove forests have been managed for ecotourism and the cultivation of fish and shrimp (Kairo *et al.*, 2001).

### **1.3.2 Mangrove replanting in Malaysia**

Before the December 26, 2004 tsunami event, mangrove ecosystems in Malaysia were rehabilitated mainly for charcoal, firewood, pole production and maintenance of an ecosystem that supports a flourishing fishing industry and a variety of wildlife species (Shamsudin *et al.*, 2008). When the tsunami struck parts of the west coast Peninsular Malaysia and caused the death of 75 people, the tragedy raised immediate awareness of the importance of planting trees in protecting coastal areas from tidal waves (Havanond, 2005). As a result, the Ministry of Natural Resources and Environment of Malaysia led by the Forestry Department Peninsular Malaysia (JPSM), Forest Research Institute Malaysia (FRIM), National Hydraulic Research Institute of Malaysia (NAHRIM), University Malaya and other governmental agencies have joined forces to combat coastal erosion, rehabilitate and conserve mangroves. The 9th Malaysia Plan (2006) had also allocated RM39 million to carry out various programmes related to conservation and preservation of coastal areas. From 2005 until 2009, the Malaysian government and various non-governmental organizations (NGOs), with the help of local communities,

had planted 5,030,057 trees at 290 locations, covering an estimated area of 1,828 hectares in the states of Perlis, Kedah, Pulau Pinang, Perak and Selangor (Mohd Ridza, 2006).

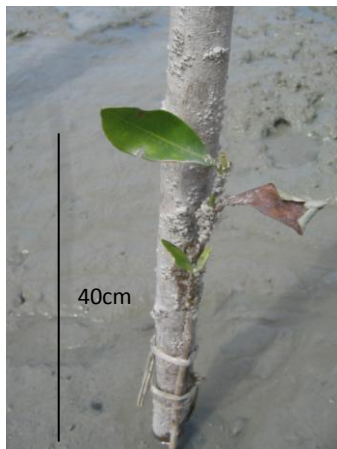
Successful replanting of mangrove trees has been a difficult task. In the site-selection for mangrove planting, numerous site characteristics should be considered, including the stability of the site, rate of siltation, soil characteristics, degree of exposure to waves and tidal currents, depth of tidal inundation, height of water table, availability of freshwater, presence of pests, availability of propagules, signs of natural regeneration (Field, 1996). In Selangor, Chan *et al.* (1988) reported that several planting trials of *Avicennia officinalis* on an exposed mud flats seaward of an eroding shoreline were not successful. Among the factors adversely affecting survival and growth of mangrove plants are strong wave actions, high soil salinity, barnacle infestation, prolonged inundation and lack of tidal flow.

To initiate the protection of replanted mangrove seedlings and to stabilize the soil, the Drainage and Irrigation Department (DID) had first installed four geotubes, each of 50m length x 3.7 m breadth x 1.8 m height, and separated by a gap of 0.5m at Kampung Sungai Haji Dorani (KSHD), Selangor, in 2006. The Forest Research Institute of Malaysia (FRIM) was appointed the lead agency to lead the mangrove rehabilitation programmers at KSHD. FRIM has been involved in research and development activities relating to the conservation and management of forests including mangrove forests. FRIM started replanting *Rhizophora apiculata* ('bakau minyak'), *Rhizophora mucronata* ('bakau kurap') and *Avicennia alba* ('api-api') mangrove trees at KSHD in 2007. The techniques used by FRIM in mangrove replanting were

conventional, comp-pillow, comp-mat and the Bamboo Encasement Method (BEM). The conventional method is one of the mangrove planting methods commonly used in mangrove replanting areas. A two meter long wooden stake or bamboo was tied to the middle part of the stem of the mangrove seedling (Figure1.1). The wooden stake was then driven 1m deep into the soil and the upper part above the mud was tied closely to the mangrove seedlings. The stake would support the seedling and prevent it from being washed away by the currents. The roots and the lower part of the stem (2-3cm) was tied to the stake using coconut fiber. This design aimed to help the replanted seedling to adapt to the problems of anaerobic soils; by storing water and ensuring the roots of the seedling were not exposed if the mud level dropped to 1 to 3cm.

The comp-pillow planting technique was developed using a coir-log as a planting case or planting medium (Figure 1.2). The coir-log was made of 100% biodegradable, compressed coconut fibre. It is encased with a high tensile 100% biodegradable coir twine netting or polypropylene (PP) netting. Each coir-log size measured 100 cm length by 30 cm height. The coir-log was punched with five holes for planting the mangrove seedlings. The pre-planted mangrove seedlings were raised in a nursery or wet nursery for at least one month before planting on the mud-flat to allow the development of mangrove roots within the coir-log. Comp-mat planting technique was developed using an open wire mesh rectangular box (100 cm x 25 cm x25 cm) as a planting case and filled up with mixed loose coconut fibre and mud as planting media (Figure 1.3). The upper part and sides of the casing were covered with polypropylene material to protect the planted seedlings from being washed away. Five cuts were made on the upper casing to allow for the planting of 5 mangrove seedlings. The BEM planting technique is a technique modified from the Riley Encasement Methods (REM) which was introduced by Bob Riley (Riley, 2005). A local bamboo species,

*Gigantochloa scortechinii* (buluh semantan), was used as a planting casing in BEM (Raja Barizan *et al.*, 2008; 2010) instead of PVC in REM.



**Figure 1.1** Conventional planting method    **Figure 1.2** Comp-pillow planting technique



**Figure 1.3** Comp-mat planting technique

### 1.3.3 Mangrove replanting in other countries

The 2004 tsunami had similar impact on other Asian countries. Indonesia pledged \$22 million and have already planted 300,000 seedlings in Aceh. In Aceh, over 10,000 ha of mangroves have been planted by the Board of Rehabilitation and Reconstruction of the Ministry of Forestry in areas affected by the tsunami (Triswanto, 2006). The

government of India has pledged \$8 million to supplement an on-going programme to rehabilitate mangroves damaged by cyclones (Check, 2005). According to UNEP (2007), 30 million mangrove seedlings covering 27,500 ha had been planted in Aceh since the 2004 tsunami. Unfortunately, most of the mangroves were planted in damaged pond areas and many seedlings were destroyed by the heavy machinery used in repair work. Other mangrove planting areas were destroyed by the construction of infrastructure, suggesting a lack of coordination among the various actors. At Keezhathottam and Velivayal, India, mangroves have been established after careful study on soil quality, species suitability, natural recruitment, land elevation, water sources, grazing effect and land-use (Balaji & Gross, 2006). The villagers have been recruited for excavation of water channels, fencing, seed collection, plantation and maintenance. More than 10,000 mangrove seedlings have been planted and 4,000 saplings have been raised in nurseries. It was reported that nursery-raised seedlings showed higher survival rates than direct planting of propagules (Balaji & Gross, 2006).

In Sri Lanka, seeds of *A. marina* can be raised by three methods, namely, deep pitting, trenching and broadcasting. In sites with grass cover, the broadcasting method is economical and effective. The trench method is appropriate when the area has been degraded due to tree cutting and grazing. Propagules of *R. mucronata* are directly transplanted into deep pits in high lying areas (Tahir, 2008). In New Zealand, both *Avicennia marina* propagules and seedlings were used in stop-bank protection and repair at several eroding sites along the estuarine reaches of the Waihou River. Propagules were grown to 4-6 leaf stage (~20 cm in height) in 500 ml biodegradable paper cups filled with estuarine mud. They were initially watered with estuarine water on two occasions, after which tap water was used as soil salinity can slow seedling growth. Old car tyres filled with estuarine mud and anchored with wooden battens were



planted with propagules and seedlings of *Avicennia marina*. Survival rates varied from 50-90% (Maxwell & Druitt, 1992).

#### **1.4 Interactions between mangrove trees and biofouling organisms**

The unique mangrove ecosystem offers an ideal habitat for a variety of organisms. The roots provide a substratum for a variety of fouling animals, such as sponges, hydroids, anemones, polychaetes, bivalves, barnacles, bryozoans and ascidians (Ellison & Farnsworth, 1990; Rani *et al.*, 2010). On mangrove tree trunks, barnacles are found vertically distributed as a result of physical tolerance and competition with other fauna for space and feeding area. The larger acorn barnacles (*Balanus* spp., Balanidae) are found on the lower, wetter portion of the tree trunk while the smaller star barnacles (*Euraphia* sp., Chthamalidae) are found distributed in the drier zones further up the tree where *Balanus* has yet to colonize (Lim *et al.*, 1999).

Planting new mangroves is a difficult task as seedling growth is affected by fouling organisms. High density of barnacles is known to cause the mortality of the seedlings (Ellison & Farnsworth, 1990). At BanDon Bay in southern Thailand, mangroves were planted on newly-formed mud flats (Angsupanich & Havanond, 1996). Seedlings of *Avicennia alba* and *Sonneratia caseolaris* died within eight months while those of *Rhizophora mucronata* died within a year. Seedling mortality was attributed to severe infestation by barnacles and frequent immersion in seawater during high tide. Macintosh and Ashton (2002) reported that barnacles attached to mangrove seedlings in high numbers may adversely affect respiration and photosynthesis. One of the ways to reduce biofouling infestations is to choose the right species of mangroves, and planting

should be done in shallow inundated sites during high tides, or in areas that are fully exposed for at least 3-4 hours a day at low tide (Ong, 2010). Barnacles also can be scrapped off every two months, but the work is tedious and impractical (Ong, 2010).

In several mangrove replanting projects conducted in Malaysia by Forestry Department, seedlings suffered heavy mortalities, suspected to be due to biofouling by barnacles e.g. in Putatan and Lahad Datu (Sabah) (Anonymous, 2007). Biofouling has been identified as the cause of mangrove seedling mortality in several mangrove replanting programmes (Ellison & Farnsworth 1990; Ong *et al.*, 2010). Similar studies elsewhere have ascribed failure of mangrove replanting due to the barnacle problem, for examples, India (Untawale, 1993), Hong Kong (Chen & Po 1997) and Kuwait (Bhat *et al.*, 2004).

### **1.5 Problem statements**

- 1) What are the species of fouling organisms and their rate of colonization on replanted mangrove seedlings?
- 2) Do the biofoulers affect the health (survival and number of leaves) of mangrove seedlings?
- 3) How can one prevent or control biofouling on mangrove seedlings?

## **1.6 Significance of study**

- (1) The proposed study will help explain the barnacle problem which is currently a bane to several government-initiated mangrove rehabilitation programmes in the country. This is because the barnacle problem if not addressed will hinder present governmental and private effort to replant and rehabilitate mangrove forests which have been identified as a natural buffer to not only protect the shoreline, but also human properties and coastal communities from high waves and storm surges. The government has placed high expectations on successfully rehabilitated mangroves that will also provide various ecosystem services.
- (2) The present study will find economical and safe ways and means to prevent or control fouling on young mangrove seedlings so as to enhance their survival and growth.
- (3) Although barnacle colonization on naturally-recruited mangrove seedlings has been largely ignored in large mangrove forests, this process in rehabilitated sites is of academic interest and concern because non-local or foreign seedlings are often translocated into such 'man-made' sites devoid of the natural mangrove community.

## **1.7 Objectives**

The main aim of the mangrove replanting project is to establish a mangrove buffer zone at KSHD so as to minimize the current problem of coastal erosion that is threatening the properties of its coastal community. Should the mangrove planting programme fail due to the invasions of fouling organisms, it would result in a large waste of resources. Therefore, the present study was designed with the following main objectives:

- 1) To determine the macrofouling organisms, their mode and rate of colonization on replanted mangrove seedlings.

- 2) To assess the damage (to tissues, number of leaves and survival) on mangrove seedlings as affected by biofouling, in particular by barnacles.
- 3) To determine the measures to prevent or control biofouling.

### **1.8 The scope of study**

The term biofouling in this study refers to the unwanted organisms that are attached on to replanted mangrove seedlings at the intertidal zone. The biofouling organisms are either sessile or mobile organisms found on the surface of mangrove seedlings. Examples of sessile organisms include barnacles and mussels and mobile organisms in particular snails.

The mangroves seedlings used in this study were *Avicennia marina*, *Rhizophora apiculata* and *Rhizophora mucronata*. The study sites were situated in Kampung Sungai Haji Dorani (KSHD) and Kampung Sungai Limau (KSL), which are located between Sabak Bernam and Sekinchan in the state of Selangor.

This research was carried out from January 2008 to November 2010. During this period the following were studied : physical environment, biofouling organisms found on replanted mangrove seedlings, the colonization and effects of biofoulers on *Avicennia marina* and *Rhizophora apiculata* (experiment 1 and 2), physical damage caused by biofoulers, the effects of removal of biofouling organisms on the growth and survival of replanted *Avicennia marina*, *Rhizophora apiculata* and *Rhizophora mucronata* seedlings (experiment 3), physical barrier to reduce biofouling organisms on replanted *Avicennia marina* (experiment 4), the effectiveness of elevated and short stake

planting methods in reducing biofouling on *Avicennia marina* seedlings (experiment 5), effects of biocidal chemicals on biofouling organisms and *Avicennia marina* seedlings (experiment 6), effects of different biocidal concentrations on fouling of *Avicennia marina* seedlings (experiment 7) and the impact of using biocidal treatments on the animals at the study site (experiment 8).

## CHAPTER 2: MATERIALS AND METHODS

### 2.1 Study area

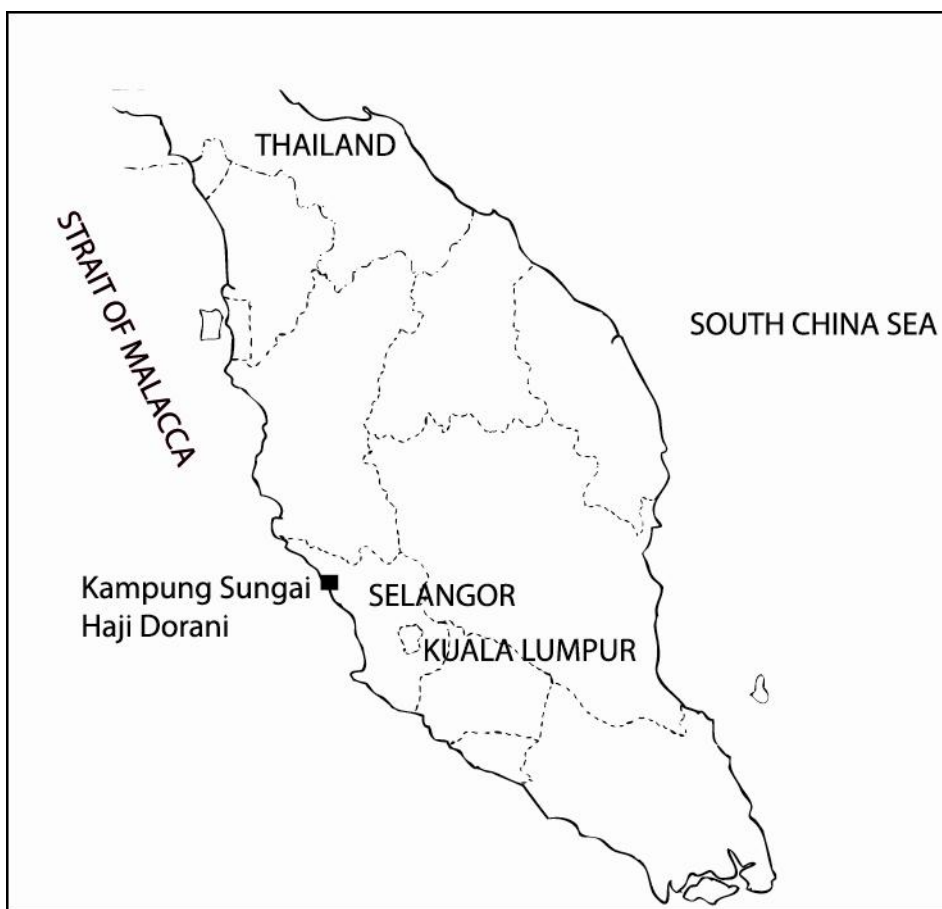
The study was carried out on the beach at Kampung Sungai Haji Dorani (KSHD: N03°38.354', E101°00.931') and Kampung Sungai Limau (KSL: N03°65.200', E 100°99.976'; Figure 2.1). The sites were located on the northwest coast of Selangor state, on the west coast of Peninsular Malaysia. The coastline is fringed by narrow sand-mud beaches and extensive mudflat areas. The mud is a mixture of clay, silt, sand and broken shells. The sediment is soft and water logged. Tidal range on spring tide was 0-3.1m, and neap tide was 1-2.3m and mean high water level was 2.5m above chart datum (Tide Tables Malaysia 2008; Figure 6.1). The annual rainfall, diurnal temperature and relative humidity were reported to be about 130 mm, 24–32°C, and 70–95%, respectively (Jeyanny *et al.*, 2009). KSHD was the site of a mangrove replanting project undertaken by the Federal government beginning in 2007.

At KSHD, a 70 m long geotube was installed on the mud flat by the Drainage and Irrigation Department (DID) in 2006 and an L-Block Breakwater (Roslan, 2006) by University of Malaya (UM) in 2008, both to act as wave breakers and to stabilize sedimentation on their leesides. The wave breakers sheltered the *Avicennia* and *Rhizophora* seedlings that were planted using different techniques by Forest Research Institute Malaysia (FRIM). A few holiday resorts and fishing landing jetties are also found in the vicinity of the study site in KSHD.

At KSL, mangroves trees that fringed the shore were *Avicennia marina* (about 2-3m tall), *Bruguiera* sp. (about 1-2m tall) and some mixed species of shrubs distributed at

an adjacent sandy beach. Relatively high density of biofouling organisms such as barnacles (*Amphibalanus amphitrite* and *Euraphia whithersi*) were found on the surface of roots and lower stems of *Avicennia marina*. Gastropods such as *Littoraria scabra* and *Littoraria melanostoma* were also found on the trunks and leaves of *Avicennia marina*.

One of the reasons KSHD was selected as the study site was due to the relatively high abundance of biofouling organisms such as barnacles that attached onto mangrove seedlings at the FRIM's Mangrove Replanting Project Site. In addition, the environmental condition at KSL is similar to KSHD. The distance between KSHD and KSL is about 3 km. Experiments in KSL had to be conducted away from the FRIM site to avoid possible contamination by tested biocide chemicals. Experiments 1, 2, 3, 4 and 5 were carried out at KSHD; experiments 6, 7 and 8 were carried out at KSL.



**Figure 2.1** Study sites at Kampung Sungai Haji Dorani in Selangor, west coast of Peninsular Malaysia.

### **2.1.1 Determination of beach profile at study site (Kampung Sungai Haji Dorani)**

Marked (in cm) PVC pipings of 2m were laid on the southern end of the geotube at KSHD. The pipes were driven into the mudflat at perpendicular distances of 10m, 30m and 50m from a marker stake on the dry beach (approximately at mean high water level). The tidal height during high tide was then marked on the pipes at a selected day and specific time. The measured tidal height indicated the height above chart datum at Kuala Bernam after corrections for tidal differences and mean heights with respect to the nearest primary port at Port Klang (100km due south) as reported in the Tide Tables for Malaysia (2008). Kuala Bernam is listed as the secondary port in the tide table, and is located about 18km north of the study site. The heights of the water level above substrate at each piping were measured at about the same time (within 5 min) and these were taken to construct the beach profile at the study site.

## **2.2 Study on the diversity of biofoulers**

Biofouler in this study was defined as the sessile or non-sessile animals found on the leaf, stem or root of replanted mangrove seedlings. A small knife was used to collect barnacles from seedlings, without damage to underlying tissue. The fouled plants were brought back to lab for identification. All species of biofouling organisms found on replanted mangrove seedlings at KSHD and KSL were identified to the lowest taxa possible under a stereo microscope. Samples of barnacles and mussels were sent to National University of Singapore for identification and confirmation of species by Dr. Tan K.S. and Dr. Serena Teo. References used to identify diversity of biofoulers included Chan and Lee (2007), Sasekumar (1974), Foster (1967), Clare and Hoeg (2008), Reid (1986) and Ockelmann (1983).

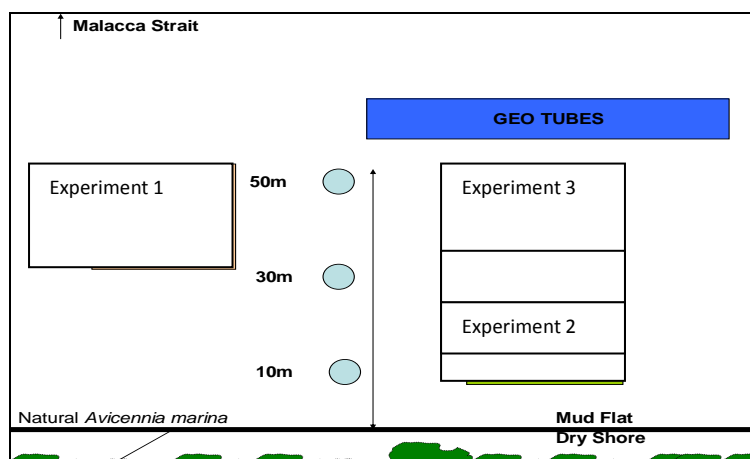


## 2.3 Study on colonization and effects of barnacles on *Avicennia marina* seedlings (Experiment 1)

This study examined the species composition, abundance, biomass, vertical distribution and cover of macrofouling assemblages on replanted *Avicennia marina* seedlings for a period of 8 weeks. The growth of seedlings was examined by the number of leaves they bore.

### 2.3.1 Experimental design and sampling

The experiment was conducted at KSHD (Figure 2.2), from May 2008 to July 2008 (8 weeks).



**Figure 2.2** Study area at Kampung Sungai Haji Dorani showing study sites of experiment 1, 2 and 3, with respect to the dry shoreline and geotube (blue). Circles indicate distance from shoreline.

All seedlings used were *Avicennia marina*, with heights that ranged from 40-50cm. The seedlings were bought from a mangrove nursery farm in Matang, in the state of Perak. All biofouling organisms had been removed before seedlings were planted into the mud.

A total of 200 *Avicennia marina* seedlings were planted 1.6m-2.0m above chart datum, 30-50m from the beach marker. Seedlings were planted in 20 rows parallel to the

shoreline, with each row having 10 seedlings each. Distance between rows was 1m. Seedlings were planted by using the conventional method (Figure 2.3). The conventional method is one of the mangrove planting methods commonly used in mangrove replanting areas. A two meter long wooden or bamboo stake was driven about 1m deep into the soil with the stem of the plant tied at its mid-region to the stake. The stake would support the seedling and prevent it from being washed away by the currents. The main root and the lower part of the stem (2-3cm above soil) was tied to the lower end of the stake using coconut fiber. This planting method allows the replanted seedling to adapt to the problems of anaerobic soils by keeping water and ensuring the roots of the seedling are not exposed if ever the mud level drops below the planted level.

Each week, ten seedlings were randomly sampled using a random numbers table to study the barnacles.



**Figure 2.3** *Avicennia marina* seedlings were planted using the conventional method at KSHD.

### 2.3.2 Soil water parameters

Temperature (°C), salinity (ppt), pH and dissolved oxygen (DO) (mg/L) were measured weekly using YSI Model 556 MPS (Multipurpose system) meter. These measurements were made to check on the soil water parameters, for example whether they could be

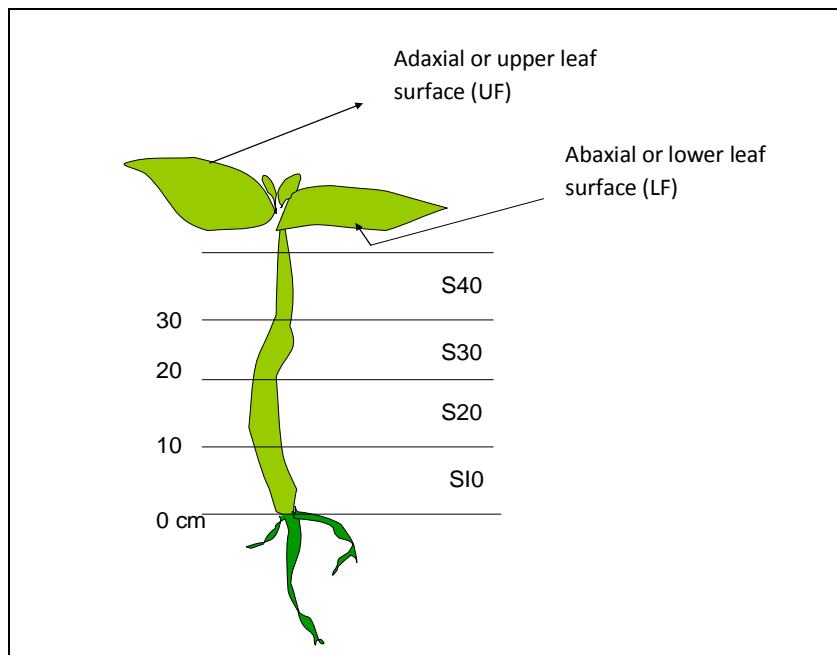
detrimental to the seedlings during exposure. The water parameters were measured during ebb tide by digging a 10-cm deep hole into substrate and allowing the interstitial water to fill it. Three replicate readings were taken from each collection site.

### 2.3.3 Laboratory treatment and analysis

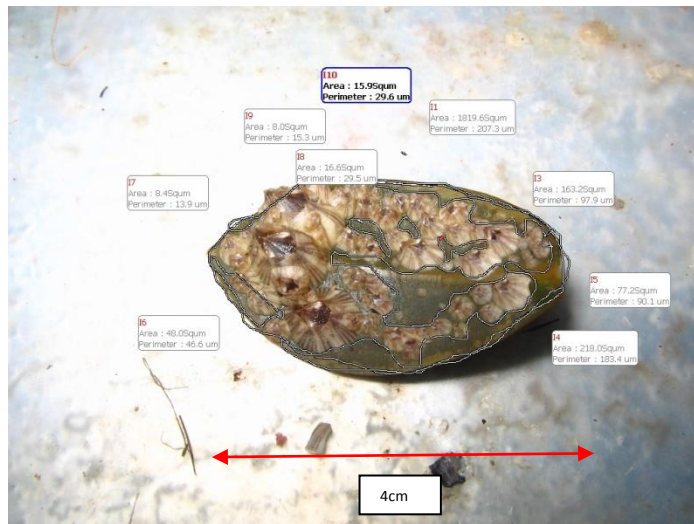
Laboratory work was carried out in the Environmental Laboratory (B201), IPS, University of Malaya.

#### 2.3.3.1 Estimation of abundance, coverage and diameter of barnacles.

Estimation of barnacle abundance and cover were made at every 10cm intervals of the stem from the height above ground (HAG) to both the abaxial and adaxial surface of the leaves (Figure 2.4). Number of individuals, coverage and diameter of barnacles were estimated with the aid of an image analyzer system (Motic Image Plus 2.0) attached to a compound or stereo microscope (Figure 2.5).



**Figure 2.4** Scoring of barnacle abundance and cover was made according to section of plant from bottom to top of stem and leaf surfaces. S10 to S40 refers to 10cm sections.



**Figure 2.5** The percentage cover of barnacles was measured by Motic Images Plus 2.0

## 2.4 Study on colonization of barnacles on *Rhizophora apiculata* seedlings (Experiment 2)

This study examined species composition, abundance distribution and percentage cover of macrofouling assemblages on newly replanted *Rhizophora apiculata* seedlings during 9 weeks. The growth of seedlings was examined by the number of leaves.

### 2.4.1 Experimental method and sampling

The experiment was conducted at KSHD, (Figure 2.2) in FRIM's mangrove replanting project site from May 2008 until July 2008 (9 weeks).

All seedlings used were *Rhizophora apiculata*, with heights that ranged from 40-50cm. All the biofouling organisms were removed before the seedlings were planted into the mud.

*Rhizophora apiculata* were planted by FRIM behind the geotube and the distance from the marker was about 10m, at 2.3m above chart datum. Seedlings were planted on the coir-log (Figure 2.6). Each week, ten seedlings were randomly sampled for barnacle study.

#### 2.4.2 Estimate number and coverage barnacles.

The methods used to estimate the number and coverage of barnacles were as described in 2.3.3.1.



**Figure 2.6** *Rhizophora apiculata* seedlings were planted on the coir-log at KSHD (experiment 2). The coir-log was made of 100% biodegradable natural compressed coconut fibre. Each coir-log size was 100 cm length and 30 cm height (covered in mud). Mangrove plant in picture approximately 50 cm tall.

### 2.5 Physical damage caused by biofoulers

Physical damage on the replanted mangrove seedlings were assessed based on observations made on their morphology. Photographs were taken using a high megapixel digital camera for further examination.

Histological study was made to see whether the barnacles had damaged the internal structures of the mangrove leaf and stem.

#### 2.5.1 Procedures

*Avicennia marina* leaves and stems from fouled plant (five replicates) were sampled from replanted mangrove seedlings for fixing, sectioning, staining and mounting.

**1. Fixing :** Leaf specimens to be sectioned were cut into the required size and then

placed in a glass container with Alcohol-acetic acid-formalin (AAAF) solution. The air was then pumped out from the container and the specimen left for 48 hours. The AAAF solution was prepared by using 90ml of ethyl alcohol, 5ml glacial acetic acid and 5ml formaldehyde.

2. The fixed leaf specimens were then washed in 70% alcohol for a few minutes.

3. Dehydration: Specimens were dehydrated through a series of increasing concentration of tertiary butyl alcohol as shown in Table 2.1.

**Table 2.1** Summary of dehydration steps for fixing leaf specimens

Step	95% ethyl alcohol (ml)	100% ethyl alcohol (ml)	Tertiary butyl alcohol (TBA) (ml)	Distilled water (ml)	Put wax chips	Time (hour)
1	50			50		4
2	50		10	40		4
3	50		20	30		4
4	50		35	15		4
5	50		50			4
6		25	75			12
7			100			12
8			100			12
9			(New TBA) 100			12
10					Yes	12

#### **4. Infiltration:**

a) Specimens were transferred from pure tertiary butyl alcohol to a mixture of equal parts of paraffin oil and tertiary butyl alcohol and were leaves overnight.

b) After adding in wax chips, the specimens were transferred into a small container, kept in an oven (58°C), and left overnight.

- c) Approximately 1/4 of the alcohol-wax mixture was poured away and replaced with 1/4 of wax mixed with TBA.
- d) After about another two hours, approximately 1/2 of the alcohol-wax mixture was poured away and replaced with 1/2 (49°C) wax mixed with TBA.
- e) After another two hours, all liquid were poured off and replaced with 49°C wax.
- f) Four hours later, all of the 49°C wax was poured away and replaced with fresh, clean 49°C wax.
- g) All the liquid underwent pumping three times.
- h) After 12 hours, all the liquid was replaced with Paraplast Plus (melting point 56°C).
- i) After 12hours, suction was applied at 25-30 atmospheric pressure for half an hour at a temperature of 58°C. The process was repeated until all of the air within the specimen was removed and replaced by Paraplast Plus wax.
- j) Fresh and clean Paraplast Plus wax was used for embedding.

## **5. Embedding**

- a) Molten Paraplast wax was poured into a paper boat.
- b) The specimens were dipped into the molten wax and arranged in the proper position. The wax block was allowed to cool down.

## **6. Sectioning**

- a) When the wax block had cooled down, the wax block with the embedded specimen was trimmed down into a suitable size.
- b) The specimens were soaked in water for 12 hours at room temperature.
- c) The embedded stem and leaf was cut into 10-12µm thick sections using a hand microtome in air- conditioned room (18°C). Serial sections forming a wax ribbon were taken.

## **7. Mounting**

- a) A slide warmer was heated up for 20 min.
- b) A drop of egg albumen was smeared onto a clean slide.
- c) A drop of distilled water was pipetted onto the slide.
- d) A cut length of the wax ribbon was placed on top of the distilled water (with the smooth side of the wax ribbon facing downwards).
- e) The slide was then warmed on a hot plate to spread out the sectioned tissue in the wax ribbon.
- f) When fully stretched, the slide was removed and excess water was drained down by tilting and placing the long edge of the slide onto a piece of filter paper.
- g) The slides were placed on a slide tray and dried in the oven at 40°C for five days.

## **8. Staining in safranin-fast green**

- a) The tissue sections on the slide were dewaxed by using xylene for 20 minutes.
- b) The slides were passed through a mixture of xylol-alcohol (1:1 xylene:95% ethanol) for 15 minutes,
- c) The slides were sequentially transferred to 95%, 80%, 70% and 50 % ethanol. The slides were left for five minutes in each concentration.
- d) The slides were stained in Safranin for 2 hours.
- e) The slides were washed in tap water.
- f) The slides were passed through 50%, 70%, 95% ethanol in each concentration for five minutes.
- g) The slides were stained in fast green 'FCF' for 5-10 seconds.



- h) The slides were differentiated in xylol-alcohol (1:1 xylene: 95% ethanol) followed by carbol-xylol (3:1 phenol crystals:xylene) by leaving the slides for 10 minutes in each solution.
- i) The slides were cleared in two changes of xylene for 30 minutes each.
- j) The slides were mounted in Canada Balsam (1:1 Canada Balsam:xylene).
- k) The slides were dried in the oven at 40°C for four days.

The prepared tissue slides were examined by three views, under a compound microscope in 200x magnification. The morphology and histology (number of abaxial hairs and percentage thickness of palisade layer) of fouled sections were compared against non-fouled section ( Figure 4.5; Figure 4.6; Li *et al.*, 2009).

## **2.6 Effects of removal of biofouling organisms on *Avicennia marina*, *Rhizophora mucronata* and *Rhizophora apiculata* seedlings (Experiment 3).**

This study examined the effect of biofouling organisms on the number of leaves and survival (%) of three species of mangrove seedlings.

### **2.6.1 Experimental design and sampling**

Experiment was conducted at KSHD in FRIM's Mangrove Replanting Project Site (Block 1; Figure 2.2) during May 2008 until October 2008 (20 weeks).

Planted seedlings were *Avicennia marina*, *Rhizophora apiculata*, *Rhizophora mucronata*, with heights that ranged from 40-50cm. These seedlings were bought from a mangrove nursery farm from Matang, Perak. All the biofouling organisms were removed before the start of the experiment. The seedlings were planted behind the geotube at a distance of 5-10m from the sandy shore. *Rhizophora apiculata* and *R. mucronata* seedlings were planted on the Comp-mats, and *Avicennia marina* were planted on the Coir-logs.

Four types of treatments were maintained throughout the trial:

- 1) Removal of all biofouling organisms from all leaves (*leaf*)
- 2) Removal of all biofouling organisms from the stem (*stem*)
- 3) Removal of biofouling organisms from all leaves and stem (- *control*)
- 4) Non-removal of biofouling organisms (+*control*).

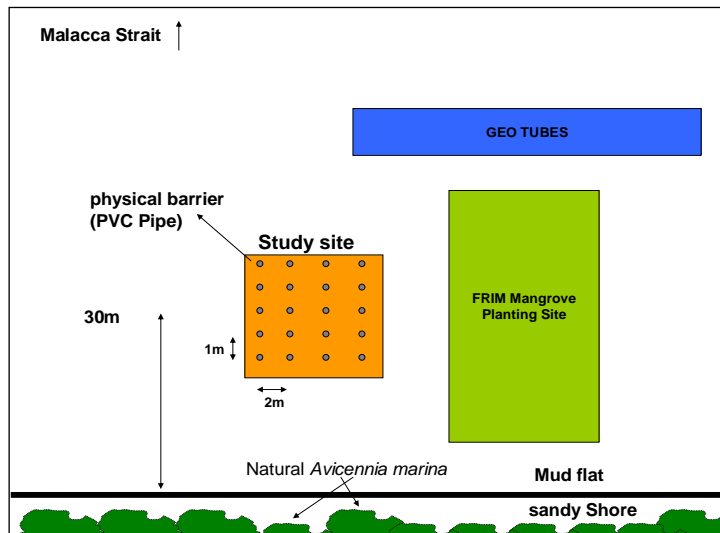
Each treatment consisted of 10 replicates. Once every two weeks, data on the number of leaves and number of survived plants were noted until the 20<sup>th</sup> week.

## **2.7 Physical barrier to reduce biofouling (Experiment 4)**

This study determined the effectiveness of using a physical barrier (PVC piping) around the plant to reduce biofouling. The abundance of biofouling organisms found on the protected seedlings and the number of leaves during 10 weeks were compared against mangrove seedlings without protection of physical barrier (i.e. using conventional method).

### **2.7.1 Experimental design and sampling.**

Experiment was conducted at KSHD from 13 April 2009. Before the experiment, tidal heights were measured to estimate the heights above chart datum. Seedlings were planted to the left of block 1 (Figure 2.7), where the height of ground was 1.8m above chart datum. All planted seedlings were *Avicennia marina* of heights of about 40-50cm. The seedlings were collected from Sarbak Bernam (2 km from the experiment site), and all biofouling organisms had been removed before planting.

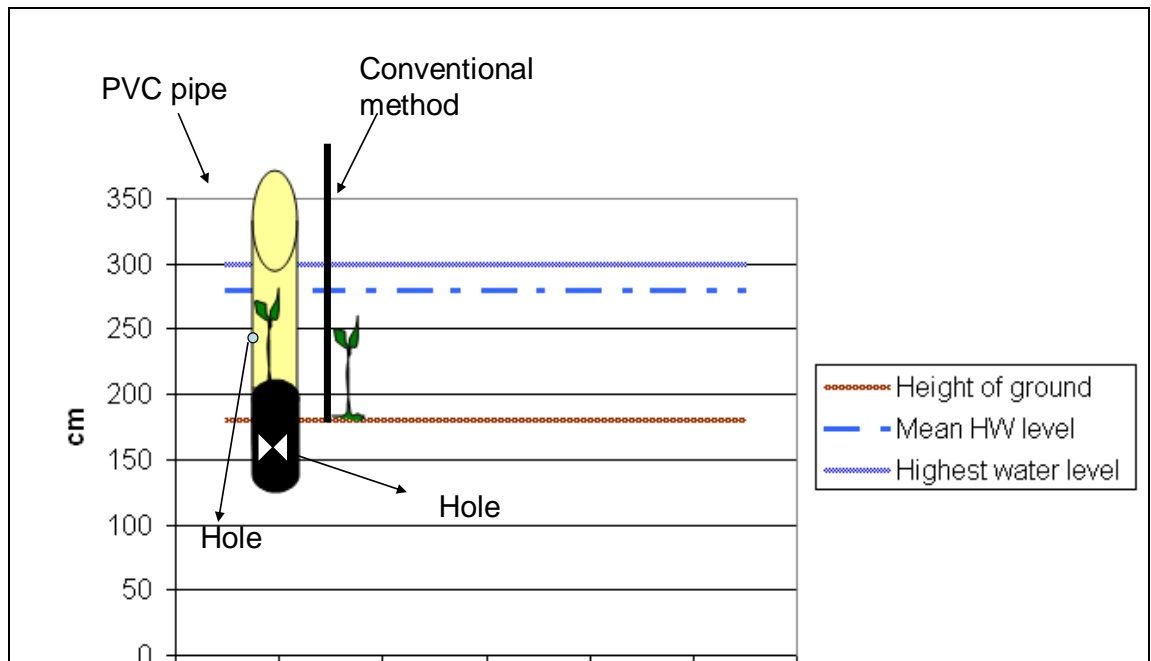


**Figure 2.7** Layout of experimental design (experiment 4) at Kampung Sungai Haji Dorani.

A PVC piping of 10cm diameter and 200cm length was used as a physical barrier as well as a planting method. A small hole with a diameter of 1cm was made at the middle portion of the PVC piping. A cloth was used to cover this hole. An additional two or three triangular openings (5cm sides) were made on the PVC piping, 0.5 m from its 'bottom' end. The piping was driven into the soil until about 100cm length of the piping remained above the mud. One seedling was then planted inside the piping with about 1/3 of its total length covered with soil at the bottom. This design allowed the roots of the growing seedling to protrude out and allowed water to seep into the tube from the bottom during high tide.

The height of the water level in the study site was about 2.7m. At times, the water level could reach up to 3.1cm during high spring tide. The height of the PVC piping was 2.8cm from ground level and thus, equals to the mean high water level (Figure 2.8). The conventional method was used as the control in this experiment. In total of 20 seedlings were planted in PVC pipings and 20 control seedlings were planted at distances of 0.5 m from the PVC pipings.

Every two weeks, three plants of each treatment were randomly sampled for examination of the abundance of biofouling organisms and the number of leaves.



**Figure 2.8** Physical barrier to reduce biofouling.

### 2.7.2 Abundance of biofouling organism

The abundance of biofouling organisms was estimated after capturing the image using a Motic camera fixed onto a compound microscope or stereo microscope.

### 2.7.3 Examination of the number of leaves

Samples of seedling were examined for their number of leaves over time.



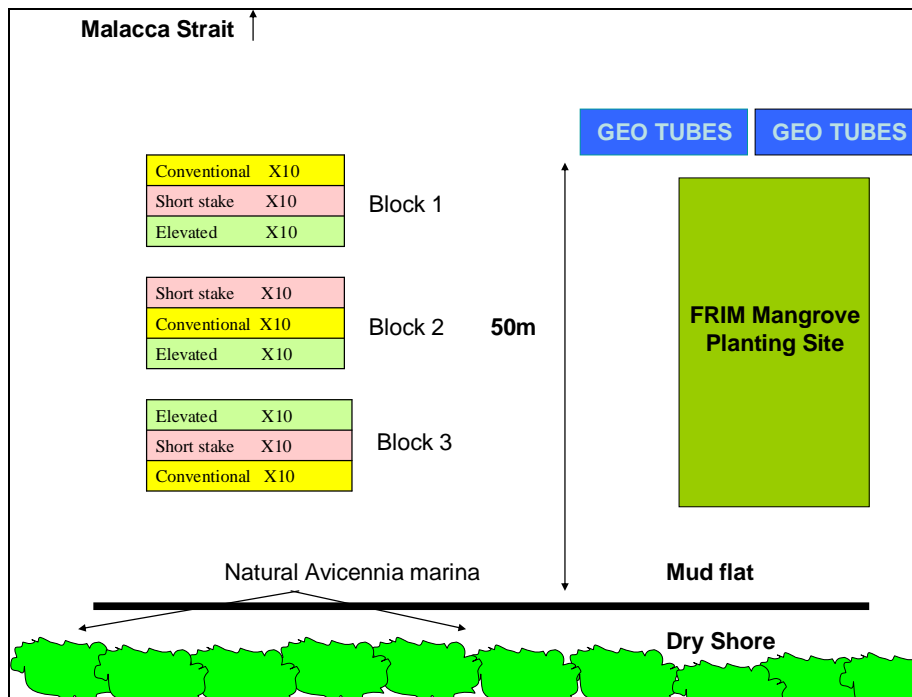
**Figure 2.9** The PVC encasement pipes in sea water during high tide. The white colour pole marked the water level above C.D.

## **2.8 Study on the effectiveness of elevated and short stake methods in reducing barnacles on *Avicennia marina* seedling (Experiment 5)**

This study examined the effect of mangrove planting method (elevated, short stake and conventional method; Figure 2.10) on the abundance of barnacles and number of leaves of *Avicennia marina*, measured each 1 week until the end of 9 weeks.

### **2.8.1 Experimental design and sampling**

The experiment was conducted at KSHD, towards the left side of the FRIM mangrove replanting site (Figure 2.8.1) from 20 January 2010.



**Figure 2.10** Experiment design to compare barnacles on *Avicennia marina* seedlings planted using three different methods. Each treatment with 10 seedlings.

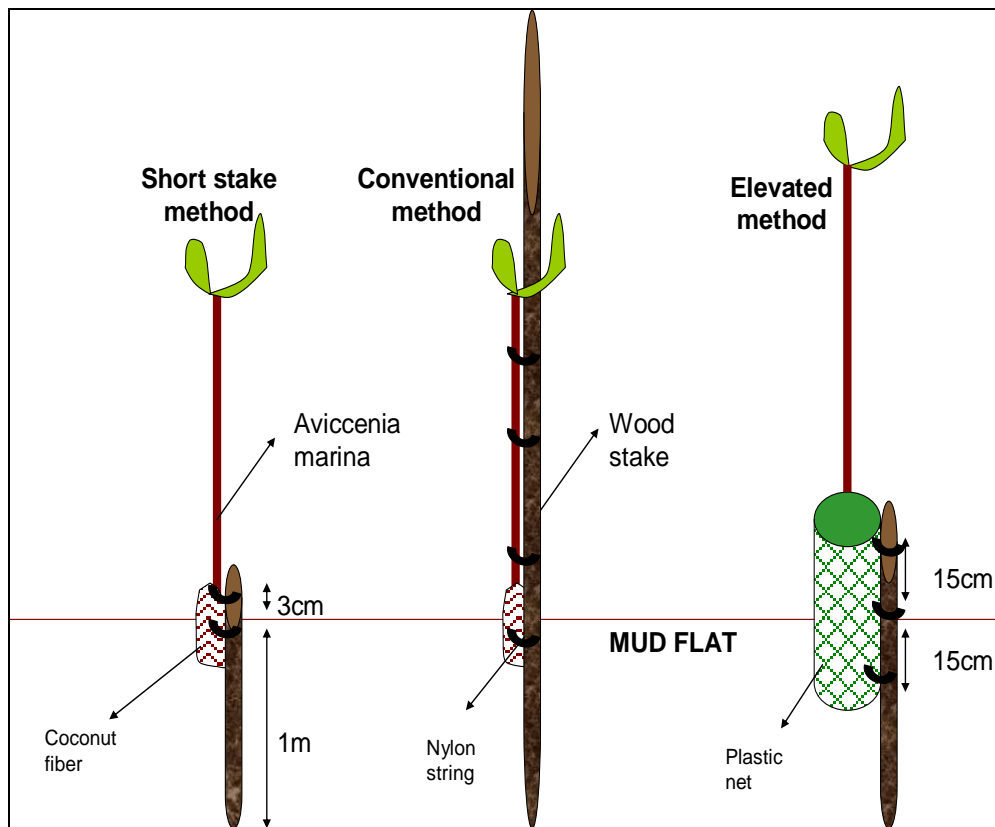
The experiment consisted of plants planted in three blocks (Figure 2.10). All three planting methods (elevated, conventional and short stake method) were present in each block. At each block, the tree planting methods, each with 10 seedlings as replicates were randomly assigned giving a total of 90 seedlings. All seedlings were *Avicennia marina*, with heights of about 40-50cm.

The conventional method is one of the mangrove planting methods commonly used in mangrove replanting areas (see Section 1.3.3).

The short stake method was designed based on the observation that wild mangrove seedlings have lesser number of barnacles attached to them as compared to seedlings planted using the conventional method (Experiment 1 and 4). The hypothesis was that the tied, non-flexible seedling (in conventional method) would allow more barnacles to attach onto it compared to short stake method. This is because the gap between the seedling and the stake has a reduced flow of the water current and thus encourages the

settlement of cyprids (Figure 6.3). Hence, the short stake method was modified from the conventional method where instead of a long stake, a short wooden stake was used as a support for the plant which was tied at its lower end (about 10cm) while leaving its upper part free to sway with the tidal movements (Figure 2.11). The main root and the lower stem (2-3cm) were tied with coconut fiber.

The elevated method was designed based on the hypothesis that a lower level of inundation by water would decrease the rate of biofouling (Experiments 1, 2, 3 and 4). The plant was thus raised up by 15cm above ground by planting it inside a cylindrical 'pot' fashioned from nylon fish nettings of 1x1cm mesh size. This aim to reduce the inundation time of elevated seedlings compared to conventional and short-stake method. The pot measured 30cm height by 10cm diameter and was stuffed with coconut fiber to hold the plant in place. The nylon net pot allowed water to penetrate and provided openings for the outgrowth of stilt roots. A short stake tied to the lower part of the net pot held it firmly in the mud (Figure 2.11). Every week, the plants of each treatment and from each block were randomly surveyed for examination of the abundance of biofouling organisms and the number of leaves.



**Figure 2.11** Mangrove planting methods (Short stake, Conventional and Elevated).



Short stake method



Elevated method

**Figure 2.12** Mangrove replanting methods

## 2.9 Effect of chemical treatments against biofouling organisms on *Avicennia marina* seedling. (Experiment 6)

This study examined the effect of chemical biocidal treatments on the abundance of biofouling organisms and the performance (number of leaves) of the planted *Avicennia marina* seedlings.



### 2.9.1 Experimental design and sampling

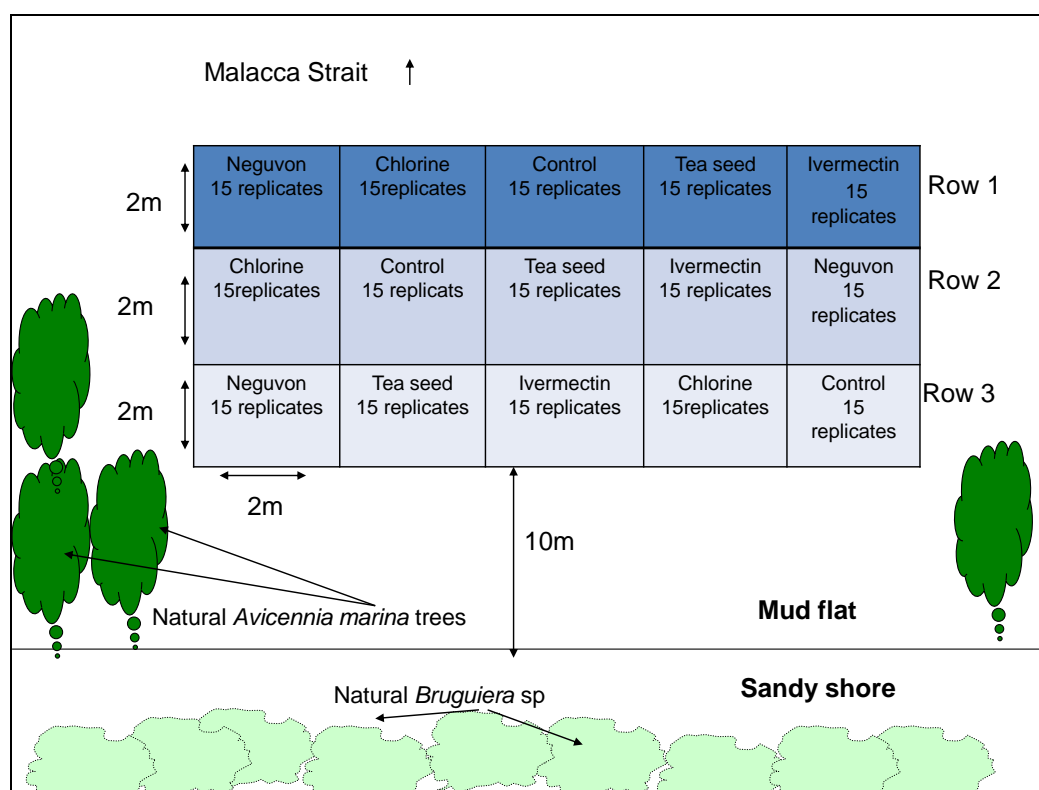
This study was carried out at the coastal area of KSL (Figure 2.13) from early September 2009 until end of October 2009 (6 weeks). All seedlings were *Avicennia marina*, with the plant height of about 30-50cm. The seedlings were planted using the conventional method.

The applied chemical biocides and their concentrations for the treatments were: tea seed cake (100g diluted in 1L distilled water), Clorox® (100ml diluted in 1L distilled water; 10% Clorox; 0.5% chlorine), Neguvon (1g diluted in 1L distilled water; 0.97mg trichlorfon/ml), Ivermectin (1ml diluted in 1L distilled water; 1µg/ml; Figure 2.14) and distilled water (control).

Tea seed cake is a residue remaining after the oil has been extracted from the seeds of certain plants in the Camellia family. Tea seed cake is extensively used in aquaculture to eliminate unwanted fishes & harmful insects in the fish and prawn /shellfish ponds (Hsiao, 2003). The active ingredient of Clorox® used was sodium hypochlorite, packed at > 5.25%. ). Neguvon is sold as a soluble powder, which can be added directly to water and is highly effective against crustacean ectoparasites and some monogeneans. The active ingredient of Neguvon used was 97% trichlorfon (Syndel, 2009). Ivermectin is an anti-parasitic drug effective against a wide variety of parasites (Burridge & Haya 1993). The concentration of Ivermectin used was 10mg/ml (1% w/v)

The chemicals were applied by painting them over the surface of leaves and stem of the seedlings using a small paint brush on a weekly basis.

The seedlings were planted in three rows. For each row, each treatment was randomly assigned to 15 seedlings. A total of 225 seedlings were planted. At each week, one seedling per treatment per block was randomly taken out from each row.



**Figure 2.13** Study site and experiment design (experiment 6) at Kampung Sungai Limau.

### 2.9.2 Soil water parameters

Salinity (ppt) and pH value were measured weekly using YSI Model 556 MPS (Multipurpose system) meter. The water parameters were measured during ebb tide by digging a 10-cm deep hole into substrate and allowing the interstitial water to fill it. Three replicate readings were taken from each collection site.

### 2.9.3 Laboratory treatment and analysis

The seedlings were brought back to the laboratory to examine the species, abundance of biofouling organisms and number of leaves.

#### 2.9.4 Estimation of abundance of biofouling organisms.

The abundance of biofouling organisms on the seedlings were estimated with the aid of Motic Images Plus 2.0, compound microscope and stereo microscope.



**Figure 2.14** The applied chemicals on *Avicennia marina* seedlings.

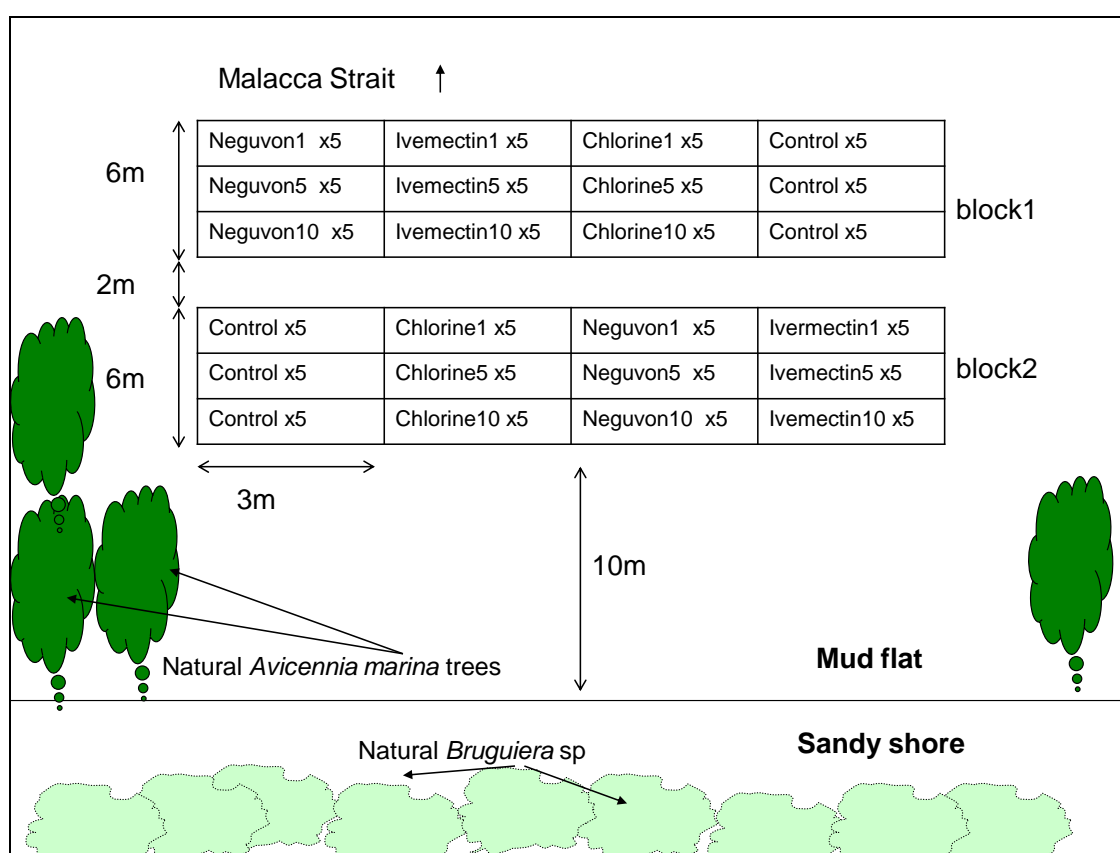
#### 2.10 Effect of different chemical concentrations against biofouling organisms on *Avicennia marina* seedling. (Experiment 7)

This study examined the effects of three chemical biocides of different concentrations and distilled water (control) on the abundance of biofouling organisms and the performance (number of leaves) of replanted *Avicennia marina* seedlings.

##### 2.10.1 Experimental design and sampling

This study was carried out at the coastal area of KSL (Figure 2.15) from 13 January 2010 until 3 March 2010 (7 weeks).

All seedlings used were *Avicennia marina*, with heights of 15-50cm. Seedlings were planted using the conventional method. This experiment had 2 blocks. In every block, each treatment (three chemicals and distilled water) was assigned to 10 seedlings as replicates. A total of 120 seedlings were planted. The treatments and the concentration chosen are listed in table 2.2. The number of biofouling organisms and mangrove leaves were counted with the aid of a hand counter during low tide, each week until the 7<sup>th</sup> week. A camera (Canon, 10 mega pixels) was used to capture the biofouling growth on the seedlings.



**Figure 2.15** Study site and experiment design. (Kampung Sungai Limau). Ivermectin1=1µg/ml of Ivermectin, Ivermectin5=5µg/ml of Ivermectin, Ivermectin10=10µg/ml of Ivermectin, Chlorine1=0.5% chlorine, Chlorine5=2.5% chlorine, Chlorine10=5% chlorine, Neguvon1=0.97mg trichlorfon/ml, Neguvon5=4.85mg trichlorfon/ml, Neguvon10=9.7mg trichlorfon/ml, control=distilled water.

**Table 2.2** The chemical treatments and concentration chosen

Treatment	Dilution	Concentration	No. of replicates
Ivermectin 1	1ml of Ivermectin (1%) mixed with 1L of distilled water	1µg/ml	10
Ivermectin5	5ml of Ivermectin (1%) mixed with 1L of distilled water	5µg/ml	10
Ivermectin10	10ml of Ivermectin (1%) mixed with 1L of distilled water	10µg/ml	10
Chlorine1	10ml of Clorox® mixed with 90ml of distilled water (10% Clorox )	0.5% chlorine	10
Chlorine5	50ml of Clorox® mixed with 50ml of distilled water (50% Clorox)	2.5% chlorine	10
Chlorine10	100ml of Clorox® (100% Clorox)	5% chlorine	10
Neguvon1	1g Neguvon mixed in 1L of distilled water	0.97mg trichlorfon/ml	10
Neguvon5	5g Neguvon mixed in 1L of distilled water	4.85mg trichlorfon/ml	10
Neguvon10	10g Neguvon mixed in 1L of distilled water	9.7mg trichlorfon/ml	10
Control	Distilled water		30

## 2.11 Effects of biocidal chemicals on other fauna at the study site (Experiment 8)

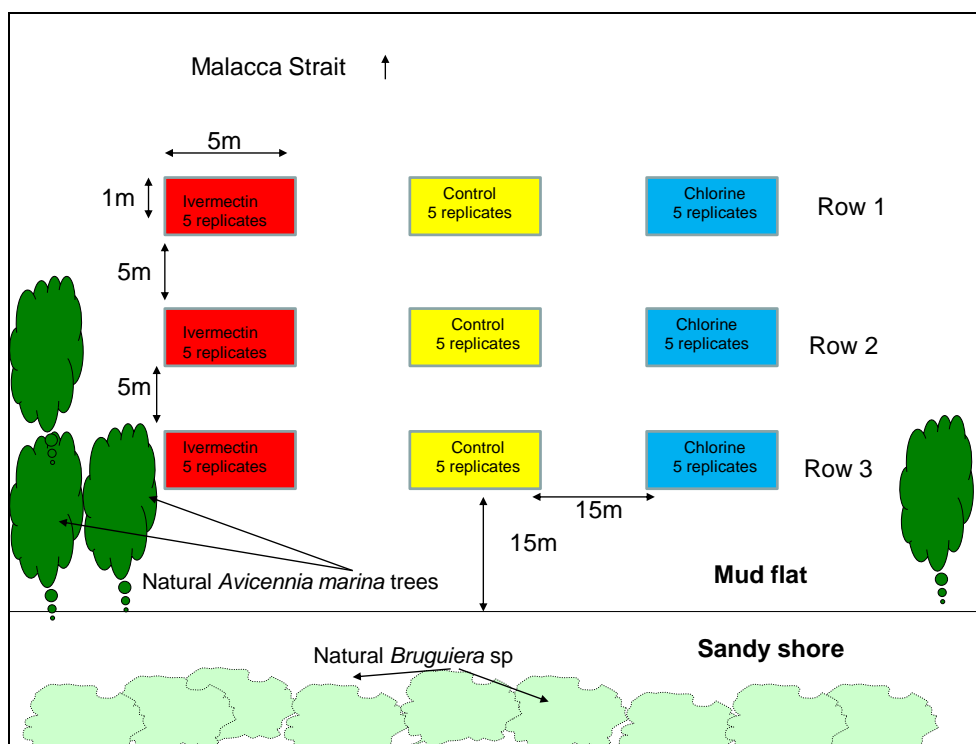
This is an extended study from the previous experiments (Experiment 6 and 7) to investigate the impact of chemical treatments namely, 2.5% chlorine (50% Clorox® mixed with 50% distilled water, v/v), 5µg/ml of Ivermectin (5ml of Ivermectin mixed with 1L of distilled water), and distilled water (control), on the population of epifauna and infauna in the study site.

### 2.11.1 Experimental design and sampling

This experiment was conducted at KSL, the same site as the previous experiments (Experiment 6 and 7), from May 2010 until July 2010 (10 weeks).

The seedlings were planted using the elevated method. The seedlings were planted in three blocks by three rows (Figure 2.16). In each row, three treatments were assigned (Ivermectin, Clorox and distilled water (control)). The distance between rows was 5m, while distance between the blocks was 15m to reduce cross-contamination effect. In each block, each treatment was assigned to five seedlings as replicates. Four wooden

stakes were driven into the sediment to form a (1m x 1m) 1m<sup>2</sup> quadrat. Seedlings were then planted at the center of the quadrat and thus, a total of 45 seedlings were planted in 45 quadrats. All seedlings were *Avicennia marina*, with heights of about 40-50cm. The treatments were brushed on to the surface of the seedlings by using a soft bristle brush during low tide every week for 10 weeks.



**Figure 2.16** Study area and experiment design (experiment 8, Kampung Sungai Limau).

### 2.11.2 Counting the population of animals in quadrat.

From a distance, a pair of binoculars was used to observe and count epifauna such as crabs and mudskippers within a 1m x 1m quadrat. For slow-moving epifauna such as gastropods, the observation was made on-site. The number of the animals found within the quadrat were counted with the aid of a hand held counter.

### 2.11.3 Counting number of infauna

To collect infauna, a cylindrical container with a height of 10.5cm, diameter of 11cm and capacity of 1L was used to dig into the mud. Five samples were randomly collected

at each treatment site. The samples were put into plastic bags and stored in the ice box and immediately brought back to the lab for sieving using a steel wire mesh sieve (aperture 1.5mm and 500 $\mu$ m; Figure 2.17) to collect the animals. The collected specimens were then preserved in 95% alcohol.



**Figure 2.17** Sieve (aperture 1.5mm and 500 $\mu$ m) used for sieving infauna.

#### **2.11.4 Measuring the organic matter content of soil.**

Sediment organic matter might indirectly determine the effects of the chemicals on the soil microorganisms. About 100g of mud sample was collected for organic matter content estimation.

Procedure:

- (1) The weight of an empty, clean, and dry porcelain dish (MP) was determined and recorded.
- (2) Approximate 10 gram of the mud sample was placed in the porcelain dish and the weight of dish and soil sample (MPDS) was determined and recorded.
- (3) The dish was placed in a muffle furnace. Temperature in the furnace was gradually increased to 500° C.
- (4) The weight of the dish containing ash (burned soil) (MPA) was determined and recorded.

**Data Analysis:**

(1) The weight of the dry soil (MD) was determined by

$$MD = MPDS - MP$$

(2) The weight of the burnt soil (MA) was determined by

$$MA = MPA - MP$$

(3) The weight of organic matter (MO) was determined by

$$MO = MD - MA$$

(4) The % organic matter (content) was determined by

$$\% \text{ Organic matter} = (MO/MD) \times 100\%$$

**2.12 Computation and statistical analysis (Experiment 1 to 8).**

Abundance data of fouling organisms were either transformed using either  $\log_{10}(x + 1)$  or square roots to satisfy the required parametric considerations before statistical analysis. These include normality and homogeneity of variance (Digby & Kempton, 1996; Zar, 1999). Similarly, percentage data were transformed by arcsines. All statistical analyses were performed using STATISTICA Version 10 Software Program. Significant difference was accepted by rejecting the null hypothesis at  $P \leq 0.05$ .

**Experiment 1**

Two-factor ANOVA was carried out to investigate the effects of plant sites (Lower Leaf, Upper Leaf, Stem 0-10cm HAG, Stem 10-20cm, Stem 20-30 cm, Stem 30-40cm and Stem 40-50cm) and time (week 1 to 8) on percentage cover (%) and the abundance (no./section) of barnacles on *Avicennia marina*. Tukey HSD test was used for multiple comparisons of the means when ANOVA showed significant result.



## **Experiment 2**

Similar methods used as described in Experiment 1 were used to analyze the data for *Rhizophora apiculata*.

## **Experiment 3**

One factor ANOVA was carried out to study the effects of the four treatments (Removal of all biofouling organisms from all leaves (*leaf*), Removal of all biofouling organisms from the stem (*stem*), Removal of biofouling organisms from all leaves and stem (*-control*), Non-removal of biofouling organisms (*+control*) on the number of leaves and the number of the surviving plants for each species of mangrove seedlings. Tukey HSD test was used for multiple comparisons of the means when ANOVA showed significant results.

## **Experiment 4**

A repeated measures 2-factor ANOVA was carried out to investigate the effects of planting method (barrier, conventional), and time (Week1-10) on the abundance of barnacles and the performance (number of leaves) of the replanting *Avicennia marina* seedlings. Tukey HSD test was used for multiple comparisons of means if the ANOVA showed a significant result.

## **Experiment 5**

Two-factor ANOVA was carried out to investigate the effects of planting method (Short stake, Conventional, Elevated), and block (1, 2 and 3) on the abundance of barnacles. 1 way ANOVA was used to analysis the effect of planting method (Short stake, Conventional, Elevated) on the abundance of barnacles and the number of leaves at week 1, 3, 6 and 9. One-way ANOVA was carried out to investigate the effects of

planting method (Short stake, Conventional, Elevated) to the performance (number of leaves) of the replanting *Avicennia marina* seedlings. Tukey HSD test was used for multiple comparisons of means if the ANOVA showed significant result.

### **Experiment 6**

Two-factor ANOVA was carried out to investigate the effects chemicals (tea seed cake, Clorox®, Neguvon, Ivomectin, distilled water) and time (week 1 to 6) on the abundance of biofouling organisms and the performance (number of leaves) of the replanting *Avicennia marina* seedlings. The Tukey HSD test was used for multiple comparisons of means if the ANOVA showed significant results. Dunnett test was used to test whether any of the chemical treatments could reduce biofouling by comparing each treatment mean against the control (distilled water) mean. Statistical tests were conducted using Statistica v. 10, and the null hypothesis was rejected at  $P \leq 0.05$ .

### **Experiment 7**

One-factor ANOVA was carried out to investigate the effects of different concentrations of chemicals (Clorox®, Neguvon, Ivomectin) on the abundance of biofouling organisms and the health (number of seedling leaves) of the replanting *Avicennia marina* seedlings. Tukey HSD test was used for multiple comparisons of the means if the ANOVA showed significant result. Dunnett test was used to test whether the chemical treatments and their concentrations had reduced biofouling by comparing each treatment mean against the control mean. Statistical tests were conducted using Statistica v. 10, and the null hypothesis was rejected at  $P \leq 0.05$ .

## **Experiment 8**

2 factor ANOVA was carried out to investigate the effects of different chemicals (Clorox®, Ivomectin and distill water) and time (week 1 to 10) on the population of epifauna and infauna and % organic matter content of soil. Tukey HSD test was used for multiple comparisons of means if ANOVA showed significant ( $P < 0.05$ ) result.

### **2.13 Summary of statistical treatments and tests**

Table 2.3 lists all the tested variables, factors, data transformations and statistical tests used for various experiments in the study. All tests were statistically tested at  $\alpha=0.05$  for acceptance and rejection of the null hypothesis.

**Table 2.3 Computation and statistical analysis (Experiment 1 to 8)**

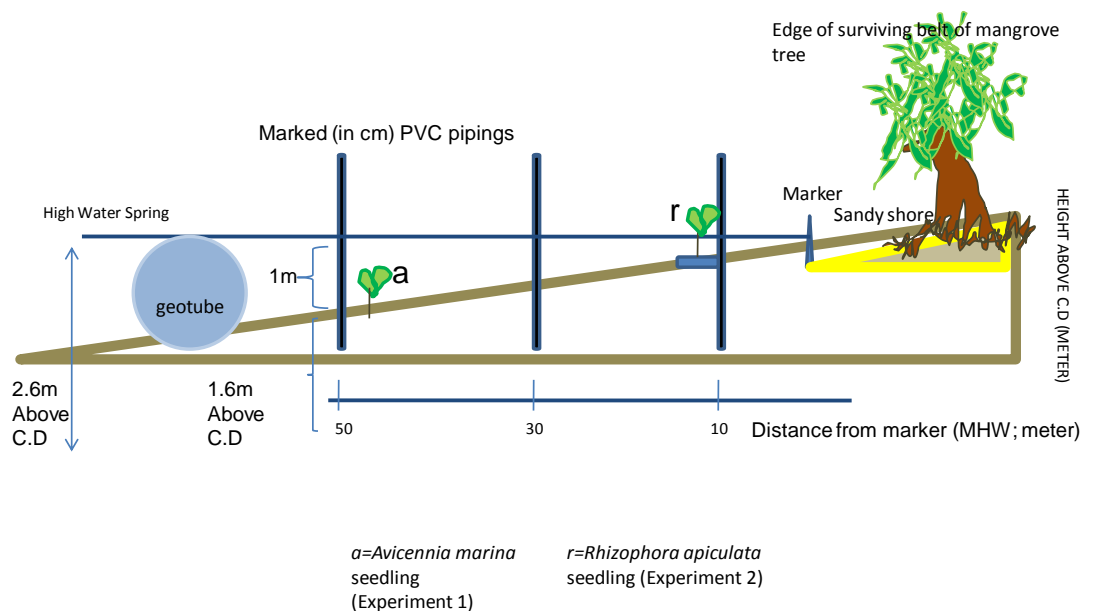
Experiment	Dependent variable	Factor	Transformation	Test
Experiment1	abundance of barnacles	segment, time	log(x+1)	2 factor ANOVA, Tukey HSD
	% cover	segment, time	arcsine	2 factor ANOVA, Tukey HSD
	number of leaves	week	none	1factor ANOVA, Tukey HSD
Experiment2	abundance of barnacles	segment, time	log(x+1)	2 factor ANOVA, Tukey HSD
	number of leaves	week	none	1factor ANOVA, Tukey HSD
Experiment 3	number of leaves	treatment	none	1factor ANOVA, Tukey HSD
	survival		none	1factor ANOVA, Tukey HSD
	number of abaxial hairs	type of leaves	none	paired t- test
Experiment 4	% thickness of palisade layer	type of leaves	none	paired t- test
	abundance of barnacles	planting method , time	none	Repeated measure 2 factor ANOVA, Tukey HSD
	number of leaves	planting method , time	none	Repeated measure 2 factor ANOVA, Tukey HSD
Experiment 5	abundance of barnacles	planting method , time, block	log(x+1)	2, factor ANOVA, Tukey HSD
	number of leaves	planting method , time	none	1, factor ANOVA, Tukey HSD
Experiment 6	abundance of barnacles	treatment	log(x+1)	1 factor ANOVA, Tukey HSD, Dunnett test
	number of leaves	treatment	none	1 factor ANOVA, Tukey HSD
Experiment7	abundance of barnacles	chemicals (code)	log(x+1)	1 factor ANOVA, Tukey HSD, Dunnett test
	number of leaves	chemicals (code), time	none	1 factor ANOVA, Tukey HSD
Exeperiment 8	abundance of epifauna	chemicals, time	log (x+1)	2 factor ANOVA, Tukey HSD
	abundance of infauna	chemicals, time	log (x+1)	2 factor ANOVA, Tukey HSD
	% content of organic matter	chemicals, time	none	2 factor ANOVA, Tukey HSD

## RESULTS

### CHAPTER 3: COLONIZATION OF BARNACLES ON REPLANTED MANGROVE SEEDLINGS.

#### 3.1 Beach profile at Kampung Sungai Haji Dorani

The range of tidal levels at Kampung Sungai Haji Doraini (KSHD) was 0-3.1m during spring tide and 1.0-2.3m during neap tide and the mean high water level was 2.5m above C.D (chart datum). The beach has a gentle gradient of 1.146°. The *Avicennia marina* seedlings used in Experiment 1 were planted at 1.6m above C.D. The *Avicennia marina* seedlings with heights of 40-50cm were flooded by the sea water during every high tide. However, the *Rhizophora apiculata* seedlings with heights of 40-50cm used in the Experiment 2 were planted at 2.5m above C.D (Figure 3.1). During certain days at neap tide, the sea water would not reach up to 2.5m above C.D.



**Figure 3.1** The beach profile at KSHD showing shore elevation with respect to chart datum( CD). Height above CD was determined from Tide Table and marked PVC poles at high spring tide.

### 3.2 Physical Environment

Water temperature in the sediment at high water ranged from 26.91°C to 32.97°C with a mean of 29.72°C± 1.75. Salinity of the water ranged from 13.26 ppt to 31.60 ppt with mean of 26.38 ppt±5.86. pH value of the water ranged from 7.43 to 8.01 with mean of 7.75±0.22. DO value of the water ranged from 2.73mg/l to 6.74mg/l with the mean of 4.33mg/l ±1.28 (Table 3.1).

**Table 3.1** Sediment water parameters (temperature, salinity, pH, DOmg/L) at Kampung Sungai Haji Dorari (May 2008 until July 2008).

week	temperature	temperature sd±	Salinity	Salinity sd±	pH	pH sd±	DO mg/L	DO mg/L sd±
mean	29.72	1.745	26.38	5.857	7.754	0.217	4.330	1.284
1	32.97	0.720	20.44	5.000	7.430	0.080	3.710	0.080
2	30.75	0.031	31.60	0.012	7.773	0.078	3.263	0.951
3	30.00	0.077	31.04	0.493	8.010	0.039	2.733	0.185
4	30.95	0.924	27.72	0.666	7.703	0.029	4.695	0.616
5	28.82	0.156	29.09	1.378	8.000	0.066	6.740	0.107
6	26.91	0.179	24.20	2.633	7.500	0.216	4.380	0.980
7	30.85	0.069	13.26	1.278	7.563	0.066	4.753	0.009
8	28.05	0.314	28.81	0.628	7.822	0.089	4.052	0.454

### 3.3 Types and description of macrobiofoulers found on replanted mangrove seedlings

#### 3.3.1 *Amphibalanus amphitrite* (Darwin, 1854)

**Scientific name:** *Balanus amphitrite* Darwin 1854 (= *Amphibalanus amphitrite* Pitombo 2004). Revision of genus name from *Balanus* to *Amphibalanus*, now known as

*Amphibalanus amphitrite* (Pitombo, 2004). This revision is due to the introduction of the new monophyletic family Amphibalanidae from the original Balanidae.

**Common name:** Striped barnacle, because its wall plates have longitudinal coloured striations.

**Classification:**

Kingdom- Animalia

Phylum- Arthropoda

Subphylum- Crustacea

Class- Maxillopoda

Subclass- Thecostraca

Infraclass- Cirripedia

Superorder- Thoracica

Order- Sessilia

Suborder- Balanomorpha

Superfamily- Balanoidea

Family- Balanidae

Genus- *Amphibalanus*

**Synonymised taxa:** *Balanus amphitrite* Darwin, 1854

**Distribution:** World-wide in warm and temperate waters. The species is the predominant barnacle of ports worldwide.

**Habitat:** The barnacles grow on all parts of the replanted mangrove seedling and sapling, but more on the roots and lower region of stems. However, they are commonly found in estuarine and coastal waters as biofouling organism on hard substrates such as rocks, seawalls, pilings, ship hulls, shoreline protective structures (e.g. riprap, geo-tube), and on living surfaces of molluscs, crabs and mangroves trees.

**Biology:** Maximum shell diameter of 30 mm. Animal with shell of six triangular, hard calcareous plates marked by distinct purple vertical stripes. The hermaphroditic animal extends out its feeding appendages to filter organic suspension during high tide. The settled cypris larva cements itself to the substrate by secreting adhesive proteins. According to Vaas (1978) and Desai et al. (2006), this species is both eurythermal and euryhaline, tolerating low temperatures (12°C) and low salinities (4 ppt), but breeding requires warmer optimum temperature of 23°C and salinities of above 15 ppt.

### 3.3.2 *Euraphia withersi* (Pilsbry, 1916).

**Common name:** Whither's barnacle

**Classification:**

Kingdom- Animalia

Phylum- Arthropoda

Subphylum- Crustacea

Class- Maxillopoda

Subclass- Thecostraca

Infraclass- Cirripedia

Superorder- Thoracica

Order- Sessilia

Suborder- Balanomorpha

Superfamily- Chthamaloidea

Family- Chthamalidae

Subfamily- Euraphiinae

Genus- *Euraphia*

**Synonym (s):** *Chthamalus withersi* Pilsbry 1916, *Chthamalus withersi* Nilsson-Cantell 1921, *Chthamalus withersi* Nilsson-Cantell 1930, *Chthamalus withersi* Zevina and Tarazov 1963, *Chthamalus withersi* Southward 1964, *Chthamalus malayensis* Pope 1965, *Chthamalus withersi* Karande and Palekar 1966, *Chthamalus malayensis* Utinomi 1968,



*Chthamalus withersi* Wagh and Bal 1974, *Chthamalus withersi* Rosell 1972, *Chthamalus withersi* Rosell 1973, *Euraphia withersi* Newman and Ross 1976

**Distribution:** Indo-West Pacific distribution. In Australia Wither's Barnacle ranges from the northern Kimberly in WA to Hervey Bay in southern Qld (WA, NT, QLD). It is absent from Yampi Sound, Carnarvon and Shark Bay, WA (Pope, 1965).

**Habitat:** On the mangrove seedlings and saplings, they are found more on the upper parts of the plant, on the adaxial leaf surface (top) and near to the shoots. Whither's barnacles have been reported to be found in similar habitats as the striped barnacles, but on the higher and drier regions of the substratum, usually between high- to mid-tide levels. They are found on coastal hard structures, river mouths and mangrove swamps.

**Biology:** Shell diameter of 7-13mm, height 4-5 mm; 5-10mm on mangrove samplings. The roughly oval body has a wavy outline formed by six flattened capitulum plates that have few, broadly jagged and ribbed bases. The dorsal opercular plates of the orifice are diamond-shaped. The suture lines of these plates are straight distinguishing it from *Chthamalus malayensis* which are wavy. Plate colour is brown, light grey to ash grey. Unlike *Balanus amphitrite* which can settle and pile up on top of each other, *E. withersi* settle close to but rarely on each other. The species is apparently tolerant of salinity and turbidity fluctuations. According to Yan & Chan (2001), the larval development of chthamaloids from nauplius to cypris stage just before settlement is about 2 weeks at 28°C.

### 3.3.3 *Fistulobalanus patelliformis* (Bruguère, 1789)

**Common name:** barnacles

**Classification:**

Kingdom- Animalia

Phylum- Arthropoda  
Subphylum- Crustacea  
Class- Maxillopoda  
Subclass- Thecostraca  
Infraclass- Cirripedia  
Superorder- Thoracica  
Order- Sessilia  
Suborder- Balanomorpha  
Superfamily- Balanoidea  
Family- Balanidae  
Subfamily- Amphibalaninae  
Genus- *Fistulobalanus*

**Distribution:** Southwest India, Thailand, Malaysia, Indonesia to Philippines.

**Habitat:** At Sungai Haji Doraini, Selangor, Malaysia, the species is found on replanted *R. mucronata* seedlings obtained from Matang mangrove forest, Perak. This barnacle species is also reported from planted mangrove seedlings (*Avicennia alba*, *Sonneratia caseolaris* and *Rhizophora mucronata*) in intertidal mudflats, and on dead or living molluscan shells and wood up to 40 metres depth (Puspari *et al.*, 2000; Rainbow, 1989).

**Biology:** Shell diameter of up to 28.5 mm and shell height of 9.2 mm have being reported by Puspasari *et al.* (2000). The shell shape varies from cylindrical (in crowded condition) to conical or depressed (in less crowded condition). The shell is often pentagonal with ribbed radii. The prominent ribs are white in colour while the general shell surface is dirty white or in older specimens dirty brown.

### 3.3.4 *Amphibalanus thailandicus* (Puspasari, Yamaguchi & Angsupanich, 2001)

**Common name:** Mangrove barnacle, teritip

**Classification:**

Kingdom- Animalia

Phylum- Arthropoda

Subphylum- Crustacea

Class- Maxillopoda

Subclass- Thecostraca

Infraclass- Cirripedia

Superorder- Thoracica

Order- Sessilia

Suborder- Balanomorpha

Superfamily- Balanoidea

Family- Balanidae

Genus- *Amphibalanus*

**Distribution:** This species is a new record for Malaysia. So far the species has been reported from Thailand.

**Habitat:** This mangrove barnacle is a recently discovered species, with similar habitats as the striped barnacle. In fact, both species can be found on the same stem or roots of replanted *Avicennia* saplings. The specimens originally described were found on the stems and roots of *Rhizophora apiculata*, *R. mucronata* and *Avicennia officinalis* in salinities that ranged from 13-30 ppt, in southwest Thailand (Puspasari *et al.*, 2001).

**Biology:** Shell diameter of 10mm observed on mangrove saplings, but Puspasari (2001) reported sizes of up to 23.9mm. The shell is conical to subcylindrical, with smooth or ribbed (larger individuals) surface. Colour is white or pink, with thin, dark purple longitudinal stripes crossed by many horizontal reddish-brown and white lines.

Distinctively different from *A. amphitrite* which has thick dark purple lines but lacking the horizontal lines as described above, the shell of *A. thailandicus* also has white longitudinal ribs as in *F. patelliformis*. *A. thailandicus* however differs from both *A. amphitrite* and *F. patelliformis* (all three belong to the so-called *B. amphitrite* complex), by having folded or ribbed shell in adults but smooth in juveniles, as well as a few more different characters (Puspasari *et al.*, 2001).

### 3.3.5 *Limnoperla mangle* (Ockelmann, 1983)

**Common name:** Small black mussel

**Classification:**

Kingdom- Animalia

Phylum- Mollusca

Class- Bivalvia

Subclass- Pteriomorphia

Order- Mytiloida

Superfamily- Mytiloidea

Family- Mytilidae

Genus- *Limnoperla*

**Synonymised taxa:** *Xenostrobus mangle* Ockelmann, 1983

**Biology:** The thin shell of the adult is dark violet to bluish-black, while the juvenile shell has a brownish coloration. A maximum size of 13.1x7.4x5.5 mm was observed by Ockermann (1983). Sexes are separate, with individuals reaching sexual maturity at 2-3 mm shell length. Larval development is planktotrophic, and developed spats settle gregariously amongst adults.

**Distribution:** Based on described materials, the species has been recorded from Malaysia and Thailand. The type-locality or site of collection of the holotype is Jeram, Klang, west coast of Peninsular Malaysia (Ockelmann, 1983).

**Habitat:** The mussel uses its fine byssus threads to attach itself onto hard substrates such as mangrove trees, stones, concrete boulders, pilings and cage fish nettings. In young mangrove saplings, this small mytilid mussel is observed to grow amongst the thick growth of barnacles.

### 3.3.6 *Littoraria scabra* (Linnaeus, 1758)

**Common name:** Rough Periwinkle

**Classification:**

Kingdom- Animalia

Phylum- Mollusca

Class- Gastropoda

Subclass- Caenogastropoda

Order- Littorinimorpha

Superfamily- Littorinoidea

Family- Littorinidae

Subfamily- Littorininae

Genus- *Littoraria*

**Synonymised taxa:** *Buccinum foliorum* Gmelin 1791, *Buccinum lineatum* Gmelin 1791, *Helix scabra* Linnaeus 1758, *Litorina scabra* var. *punctata* Philippi 1847, *Litorina scabra* var. *rubra* Philippi 1847, *Littorina novaehiberniae* Lesson 1831, *Littorina pallescens* var. *erronea* Nevill 1885, *Littorina scabra* (Linnaeus 1758), *Littorina scabra* var. *tenuis* Nevill 1885, *Melarthaphe scabra* (Linnaeus 1758)

**Biology:** Shell 25-35mm long, ribbed, with strong peripheral keel, colour pale with dark brown or black oblique axial stripes. Sexually dimorphic, males being smaller with lower spire and larger aperture (Reid, 1986). The wide, white columella of *Littoraria scabra* distinguishes this species from other similar members of *Littoraria*. However, the most reliable character for the identification of *Littoraria* species is the shape of the penis in males which is a forked structure located on the right side of the neck of the animal. According to Reid (1986), the snail is ovoviviparous; eggs developed within the mantle cavity and released at the late veliger stage.

**Distribution:** Wide distribution in tropical and subtropical areas, largely in the Indo-Pacific region from eastern Africa to Polynesia, including northern Australia and southern Japan (Reid, 1986)

**Habitat:** Lives on the lower trunk of mangrove tree and its prop roots, as well as on the leaves (up to 2m above soil). On replanted mangrove propagules and saplings (30cm to 1m height), the snail is observed on the roots (above mud), stems and leaves, as well as on attached barnacles. Snails of the genus *Littoraria* occur on mangrove trees, and show both vertical and horizontal patterns of zonation that result from their behavioral responses and physiological tolerance (Reid, 1986). Both *Littoraria scabra* and *L. melanostoma* are mainly found on the seaward edge of mangrove forests (Berry, 1972).

### **3.3.7 *Littoraria melanostoma* (Gray, 1839)**

**Common name:** Black-mouth mangrove periwinkle

**Classification:**

Kingdom- Animalia

Phylum- Mollusca

Class- Gastropoda

Subclass- Caenogastropoda

Order- Littorinimorpha

Superfamily- Littorinoidea

Family- Littorinidae

Subfamily- Littorininae

Genus- *Littoraria*

**Synonymised taxa:** *Littorina melanostoma* Gray 1839, *Littorina melanostoma* var. *articulata* Nevill 1885

**Biology:** Shell height 20-30mm, spire tall with straight outline, colour pale yellow with brown or gray dots arranged in spiral pattern. Shell opening pale yellow with black patch. Shell opening with black edge ("melanostoma" means "black mouth"). Operculum dark brown. The animal is yellowish with short fat tentacles. The snail is apparently inactive during day, but during night, it crawls downwards. According to Lee *et al.* (2002), the snail is a generalist grazer, ingesting mangrove bark, epidermal plant cells, fungi, microalgae and cyanobacteria. The snail apparently releases egg capsules into the sea every two weeks during the spring tides.

**Distribution:** Tropical and subtropical region from east India to South-east Asia, southern China and Taiwan.

**Habitat:** The snail occurs mainly at the lower level of mangrove tree trunks up to 2m above soil, staying above the high tide level. Juveniles mainly on leaves. On mangrove propagules and saplings, the snail is found in the same regions as *L. scabra*.

**Table 3.2** Biofouling organisms found on the replanted mangrove seedlings.

Animal	Scientific name	
Barnacles	<i>Amphibalanus amphitrite</i>	(Figure 3.2a)
Barnacles	<i>Euraphia withersi</i>	(Figure 3.2b)
Barnacles	<i>Fistulobalanus patelliformis</i>	(Figure 3.2c)
Barnacles	<i>Amphibalanus thailandicus</i>	(Figure 3.2d)
Bivalve	<i>Limnoperna mangle</i> (mussel)	(Figure 3.2e)
Gastropod	<i>Littoraria scabra</i>	(Figure 3.2f)
Gastropod	<i>Littoraria melanostoma</i>	(Figure 3.2g)



**Figure 3.2a** *Amphibalanus amphitrite*, the dominant biofouler on mangrove seedlings.





**Figure 3.2b** *Euraphia withersi*



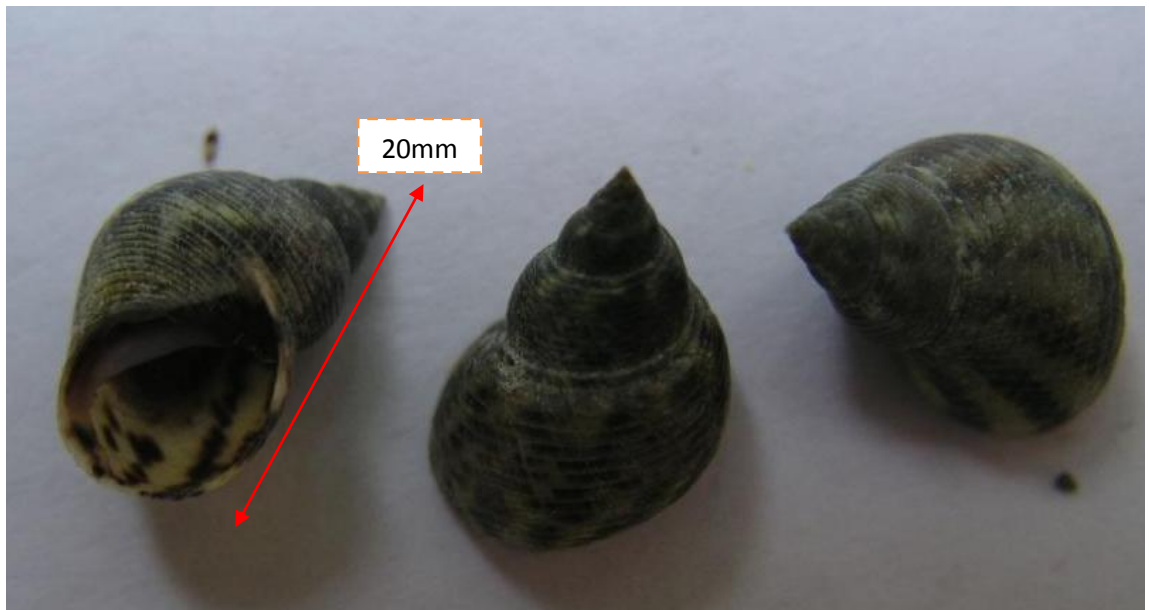
**Figure 3.2c** *Fistulobalanus patelliformis*



**Figure 3.2d** *Amphibalanus thailandicus* (dorsal view). Note *A. amphitrite* on right.



**Figure 3.2e** *Limnoperna mangle*



**Figure 3.2f** *Littoraria scabra*.



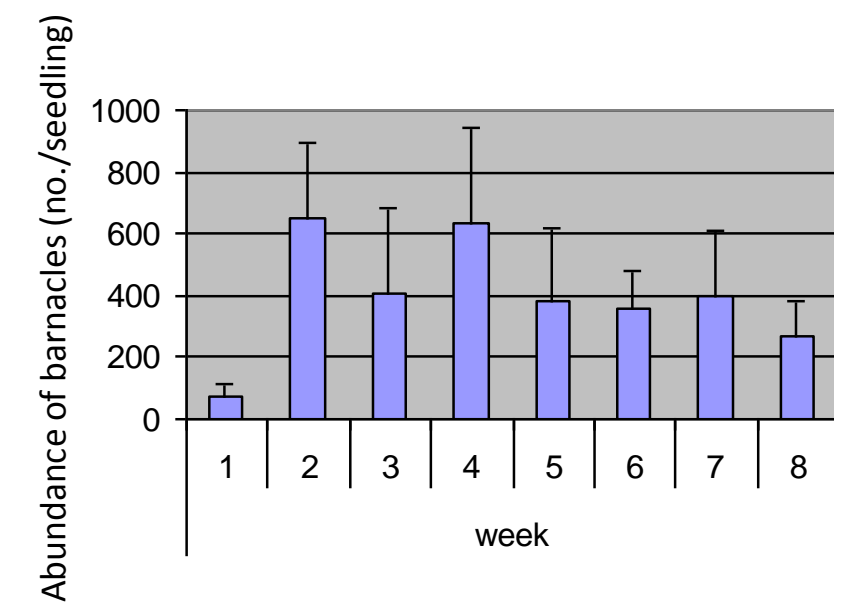
**Figure 3.2g** *Littoraria melanostoma*.



### 3.4 Colonization of barnacles on *Avicennia marina* seedlings. (Experiment 1)

#### 3.4.1 Abundance of barnacles by period of infestation

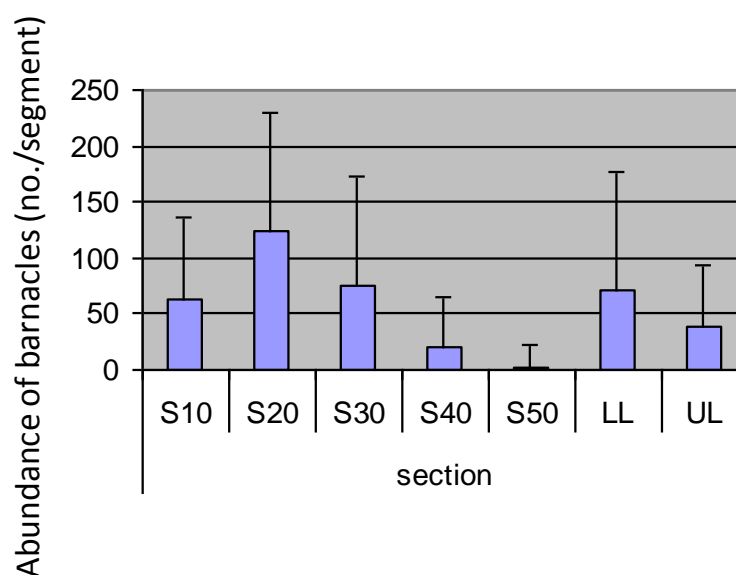
The effect of time on the abundance of barnacles was significantly different ( $p < 0.0001$ ; Appendix 1a). The mean abundance of the barnacles on *Avicennia marina* seedlings with height that ranged from 40-50cm over a period of 8 weeks was  $397.49 \text{ no./plant} \pm 269.44$ . The mean abundance of barnacles at the 1<sup>st</sup> week (73 no./plant) was significantly lower compared to others ( $p < 0.05$ ; Appendix 1b; Figure 3.3). The mean abundance of barnacles increased by 793% (651.9 no./plant) at the 2<sup>nd</sup> week. There were no significant difference among barnacles abundance at the 3<sup>th</sup> week to 8<sup>th</sup> week ( $p > 0.05$ ). Barnacles' abundance significantly increased with time. However, from the 5<sup>th</sup> week to the 8<sup>th</sup> week, the population of barnacles became relatively consistent (300-400 no./plant).



**Figure 3.3** The mean abundance (no./plant) of barnacles on *Avicennia marina* seedlings in a period of 8 weeks. Vertical bars denote standard deviation.

### 3.4.2 The abundance of barnacles on different stem segments of the *Avicennia marina* seedlings including leaf surface.

The abundance of barnacles on different sections of *Avicennia marina* seedlings showed significant difference ( $p < 0.0001$ ; Appendix 1a). The mean abundance of barnacles at S20 (stem height 10-20cm above ground; 124.79 no./segment  $\pm 105.73$ ) was significantly higher ( $p < 0.001$ ; Appendix 1c) compared to LL (lower leaf; 71.13no./segment $\pm 104.71$ ), UL (Upper leaf; 38.65no./segment $\pm 55.56$ ), S10 (stem 0-10cm height above ground; 64no./segment $\pm 73.17$ ), S30 (stem 20-30cm height above ground; 75.89no./segment $\pm 96.06$ ), S40 (stem30-40cm height above ground; 20.47no./segment $\pm 43.67$ ) and S50 (stem 40-50cm height above ground; 2.56no./segment $\pm 19.6$ ) ( Figure 3.4.). The S50 segment had the lowest abundance compared to others ( $p < 0.001$ ). Although the abundance of barnacles was higher on LL (71.12 no./ segments) compared to the UL (36.65 no./ segments), the results showed no significant difference ( $p > 0.05$ ).



**Figure 3.4** The mean abundance (no./segment) of barnacles on different segments of *Avicennia marina* seedlings. S10 (stem 0-10cm height above ground), S20 (stem10-20cm height above ground), S30 (stem 20-30cm height above ground), S40 (stem30-40cm height above ground) and S50 (stem 40-50cm height above ground), LL (lower leaf), UL (Upper leaf). Vertical bars denote standard deviation.

### 3.4.3 Interaction effect of time and segments height on the abundance of barnacles

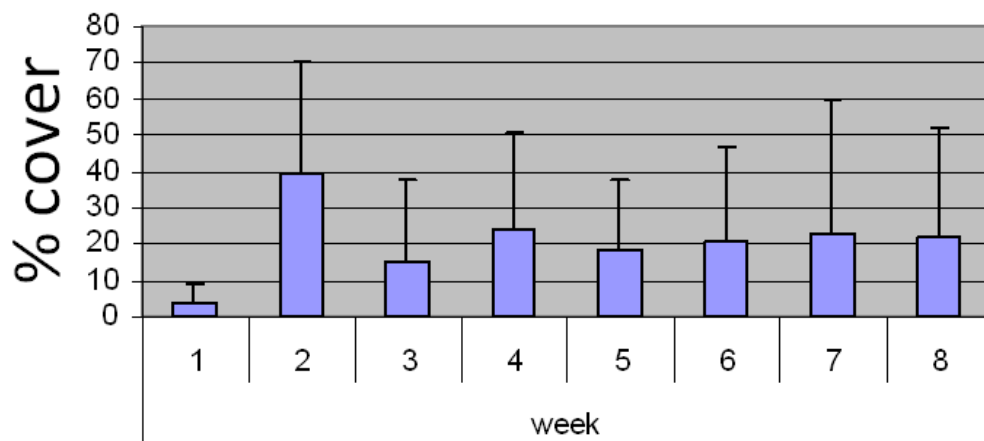
The interaction effect of time and segment on the abundance of barnacles was significantly different ( $p < 0.001$ ; Appendix 1a). The mean abundance of barnacles on S20 segment was significantly higher compared to the S50 from 1<sup>st</sup> week until 8<sup>th</sup> week ( $p < 0.05$ ; Table 3.3; Appendix 1d). The mean abundance of barnacles on the S20 segment was significantly higher compared to S30 segment from the 2<sup>nd</sup> week until the 8<sup>th</sup> week ( $p < 0.05$ ). The mean abundance of barnacles on the LL and UL from the 6<sup>th</sup> week until the 8<sup>th</sup> week was significantly lower compared to the 2<sup>nd</sup> week ( $p < 0.05$ ). The mean abundance of barnacles on the S10, S20 and S30 segment, were significantly higher compared to the LL, UL, S40 and S50 segment in the final two week ( $p < 0.05$ ).

**Table 3.3** The mean abundance (no./segment) of barnacles on different segments of the *Avicennai marina* seedlings in a period of 8 weeks. S10 (stem 0-10cm above ground), S20 (stem 10-20cm above ground), S30 (stem 20-30cm above ground), S40 (stem 30-40cm above ground) and S50 (stem 40-50cm above ground), LL (lower leaf), UL (Upper leaf).

segment/ week	1	2	3	4	5	6	7	8
LL	16.9	187.4	76.3	139.4	86.7	53.1	8.4	0.8
UL	9.7	115.3	48.8	74.8	41.4	18.7	0.1	0.4
S10	3.1	88.8	34.5	96.1	38.2	61.2	100.9	89.2
S20	14.8	125.8	95.6	218.9	105.3	112.6	216.3	109
S30	16.9	100.7	115.8	99.8	87.4	53.7	73.4	59.4
S40	11.5	33.9	34.4	14.9	20.8	41.3	0	7
S50	0.1	0	0	0	3.1	17.3	0	0

#### 3.4.4 Percentage cover of barnacles by period of infestation.

The main effect of time on the percentage cover of barnacles was significantly different ( $p < 0.001$ , Appendix 1e). The mean percentage cover of barnacles at the 1<sup>st</sup> week was 3.6 % (Figure 3.5). Percentage covered by barnacles at the 2<sup>nd</sup> week (39.51%) significantly increased compared to the others ( $p < 0.05$ ; Appendix 1f). As the abundance of barnacles increased, it contributed to the increase of percentage cover on the mangrove seedlings. However, from the 5<sup>th</sup> week to the 8<sup>th</sup> week, the percentage cover of barnacles became relatively consistent. The percentage cover of barnacles from the 3<sup>rd</sup> week (14.81%) until the 8<sup>th</sup> week (21.27%) was however not significantly different ( $p > 0.05$ ).

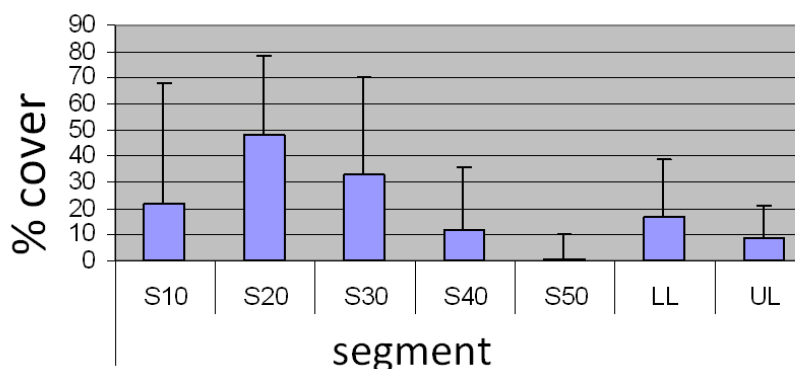


**Figure 3.5** The mean percentage cover of barnacles on *Avicennia marina* in a period of 8 weeks. Vertical bars denote standard deviation.

#### 3.4.5 Percentage cover of barnacles on different segments of *Avicennia marina*.

The percentage cover of barnacles among the different segments of *Avicennia marina* was significantly different ( $p < 0.05$ ). The mean percentage cover of barnacles on S20 segment (47.6%) was significantly higher compared to the others ( $p < 0.05$ ; Figure 3.6). The S50 segment (1.13%) was significantly lower in abundance compared to others ( $p < 0.05$ ). The percentage cover of barnacles on the S10 (22.54%) and S30 (34.45%)

segments was not significantly different ( $p>0.05$ ). Barnacles cover on the S40 (11.8%) segments was significantly higher compared to S50 ( $p<0.05$ ) segments. The percentage cover of barnacles on the LL (16.9%) was significantly higher compared to the UL surface ( $p<0.05$ ; 8.89%).



**Figure 3.6** The mean percentage cover of barnacles on different segments above ground of *Avicennia marina*. S10 (stem 0-10cm above ground), S20 (stem 10-20cm above ground), S30 (stem 20-30cm above ground), S40 (stem 30-40cm above ground) and S50 (stem 40-50cm above ground), LL (lower leaf), UL (Upper leaf). Vertical bars denote standard deviation.

#### 3.4.6 Interaction effect of time and segments height on the percentage cover of barnacles

The interaction effect of time and segment on the percentage cover of barnacles was significantly different ( $p<0.001$ ; Appendix 1e). The percentage cover of barnacles on S20 segment was significantly higher compared to the S50 from 1<sup>st</sup> week until 8<sup>th</sup> week ( $p<0.05$ ; Table 3.4; Appendix 1g). The percentage cover of barnacles on the S20 segment was significantly higher compared to S30 segment from the 2<sup>nd</sup> week until the 8<sup>th</sup> week ( $p<0.05$ ). The percentage cover of barnacles on the LL and UL from the 6<sup>th</sup> week until the 8<sup>th</sup> week was significantly lower compared to the 2<sup>nd</sup> week ( $p<0.05$ ). The percentage cover of barnacles on the S10, S20 and S30 segment, were significantly higher compared to the LL, UL, S40 and S50 segment in the final two week ( $p<0.05$ ).



**Table 3.4** The mean percentage cover (%./segment) of barnacles on different segments of the *Avicennai marina* seedlings in a period of 8 weeks. S10 (stem 0-10cm above ground), S20 (stem 10-20cm above ground), S30 (stem 20-30cm above ground), S40 (stem 30-40cm above ground) and S50 (stem 40-50cm above ground), LL (lower leaf), UL (Upper leaf).

segment/								
week	1	2	3	4	5	6	7	8
LL	4.4	55.5	5.0	30.6	15.3	19.7	3.6	1.1
UL	2.8	37.5	2.1	14.2	6.2	7.6	0.1	0.6
S10	0.4	40.1	12.9	23.8	12.6	15.3	39.5	35.7
S20	7.4	61.0	35.5	54.2	38.7	47.5	76.0	60.5
S30	5.2	66.0	31.7	28.1	28.6	29.5	42.5	44.0
S40	4.9	16.5	16.5	13.0	16.5	20.0	0.0	7.0
S50	0.1	0.0	0.0	0.0	0.5	8.5	0.0	0.0

### 3.4.7 Rate of colonization of barnacles on *Avicennia marina*.

The mean rate of colonization of barnacles on *Avicennia marina* increased at 2<sup>nd</sup> week (Table 3.5). However the rate of colonization of barnacles decreased at 4<sup>th</sup> week to 8<sup>th</sup> week.

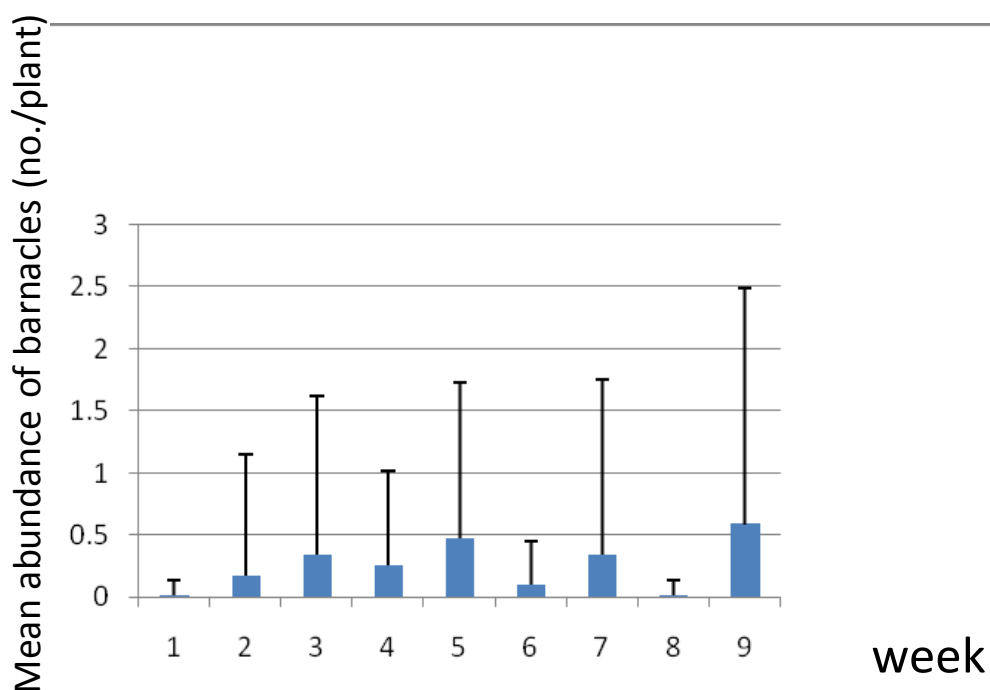
**Table 3.5** The mean rate of colonization of barnacles on *Avicennia marina* over the period of 8 weeks.

week	1	2	3	4	5	6	7	8
rate colonization (no./week)	73.00	325.95	135.13	160.98	76.58	59.65	57.01	33.23

### 3.5 Colonization of barnacles on *Rhizophora apiculata* seedlings. (Experiment 2)

#### 3.5.1 Abundance of barnacles with period of infestation

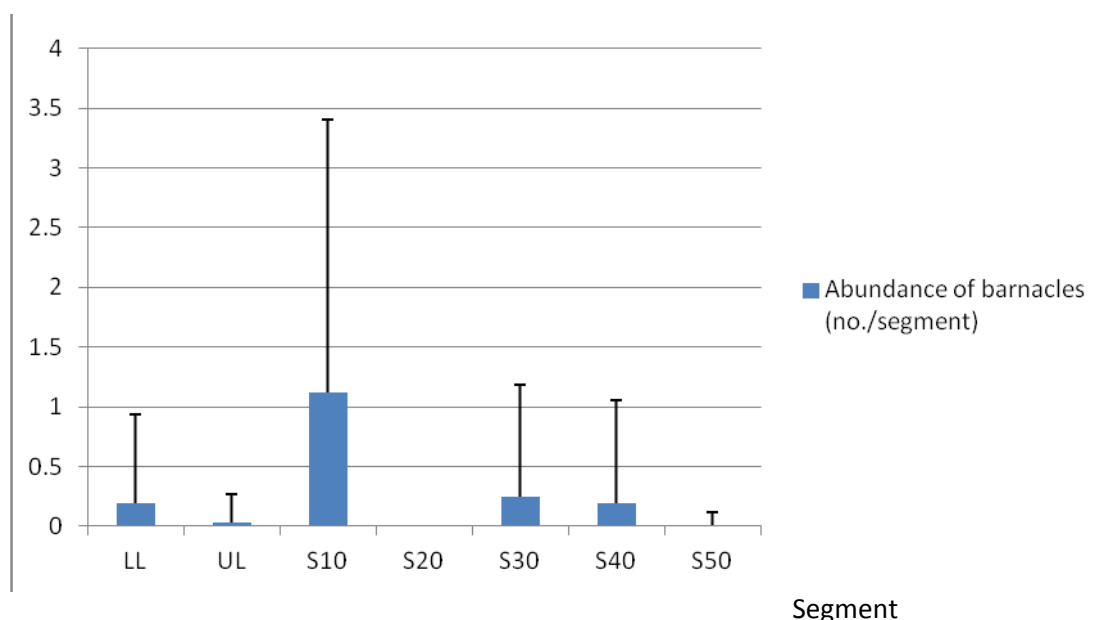
The main effect of time on the abundance of barnacles was significantly different ( $p < 0.0001$ ; Appendix 2a). The mean abundance of barnacles on the *Rhizophora apiculata* seedlings was very low with an average of not more than 5 barnacles per plant (Figure 3.7). The mean abundance of barnacles on *Rhizophora apiculata* seedlings at the 5<sup>th</sup> week (0.47 no./plant) and 9<sup>th</sup> week (0.58 no./plant) was significantly higher compared to the 1<sup>st</sup> week (0.01 no./plant) and 8<sup>th</sup> week (0.01 no./plant;  $p < 0.05$ ; Appendix 2b).



**Figure 3.7** The mean abundance (no./plant) of barnacles on *Rhizophora apiculata* seedlings in a period of 8 weeks. Vertical bars denote standard deviation.

### 3.5.2 The abundance of barnacles on different stem segments of *Rhizophora apiculata* seedlings including leaf surface.

The mean abundance of barnacles on different segments of *Rhizophora apiculata* seedlings planted at 2.5m above C.D was significantly different ( $p < 0.0001$ ; Appendix 2a). The mean abundance of barnacles on S10 segment (1.12 no. /plant) was significantly higher compared to the S20 segment (0 no./plant), S30 segment (0.24 no./plant), S40 segment (0.19 no. /plant) and S50 segment (0.01 no./plant;  $p < 0.001$ ; Figure 3.8.). There was no significant difference between ( $p > 0.05$ ) surface of leaf for LL (0.19 no. /plant) and UL (0.03 no./plant;  $p > 0.05$ ; Appendix 2c).



**Figure 3.8** The mean abundance (no./section) of barnacles on different segments of the *Rhizophora apiculata* seedlings. S10 (stem 0-10cm above ground), S20 (stem 10-20cm above ground), S30 (stem 20-30cm above ground), S40 (stem 30-40cm above ground) and S50 (stem 40-50cm above ground), LL (lower leaf), UL (Upper leaf). Seedlings were planted at 2.5m above C.D. Vertical bars denote standard deviation.

### 3.5.3 Interaction effect of time and segment height on the abundance of barnacles on *Rhizophora apiculata* seedlings

The interaction effect of time and segment height on the mean abundance of barnacles was significantly different ( $p < 0.001$ ; Appendix 2a). The mean abundance of barnacles on the S10 segment above ground was significantly higher compared to the others at the

7<sup>th</sup> week and the 9<sup>th</sup> week ( $p<0.05$ ; Table 3.6; Appendix 2d). The percentage frequency of barnacles found on S10 (89%) was highest compared to other segments of the stem. The percentage frequency of barnacles found on the lower leaf (56%) was higher compared to the upper leaf (11%).

**Table 3.6** The mean abundance (no./segment) of barnacles on the different segments of *Rhizophora apiculata* seedlings over a period of 9 weeks. S10 (stem 0-10cm above ground), S20 (stem 10-20cm above ground), S30 (stem 20-30cm above ground), S40 (stem 30-40cm above ground) and S50 (stem 40-50cm above ground), LL (lower leaf), UL (Upper leaf).

segment/week	1	2	3	4	5	6	7	8	9
LL	0	0	0.2	0.4	0.8	0.2	0	0.1	0
UL	0	0	0	0	0.3	0	0	0	0
S10	0.1	0.2	1.3	1	1.2	0.1	2.2	0	4
S20	0	0	0	0	0	0	0	0	0
S30	0	0.9	0.4	0.1	0.2	0.3	0.2	0	0.1
S40	0	0	0.5	0.3	0.8	0.1	0	0	0
S50	0	0.1	0	0	0	0	0	0	0

#### 3.5.4 The mean rate of colonization of barnacles on *Rhizophora apiculata*.

The mean rate of colonization of barnacles on *Rhizophora apiculata* was slow, which only increased from 0.1 no. /plant to the 0.2 no./plant at second week and 0.8 no. /plant at the third week. (Table 3.7). However the mean rate of colonization of barnacles decreased at 4<sup>th</sup> week. The mean rate of colonization becomes fluctuated from 5<sup>th</sup> week until the 8<sup>th</sup> week. At the ninth week, the mean rate of colonization was 0.46 no. /plant.

**Table 3.7** The mean rate of colonization of barnacles on *Rhizophora apiculata* over the period of 9 weeks.

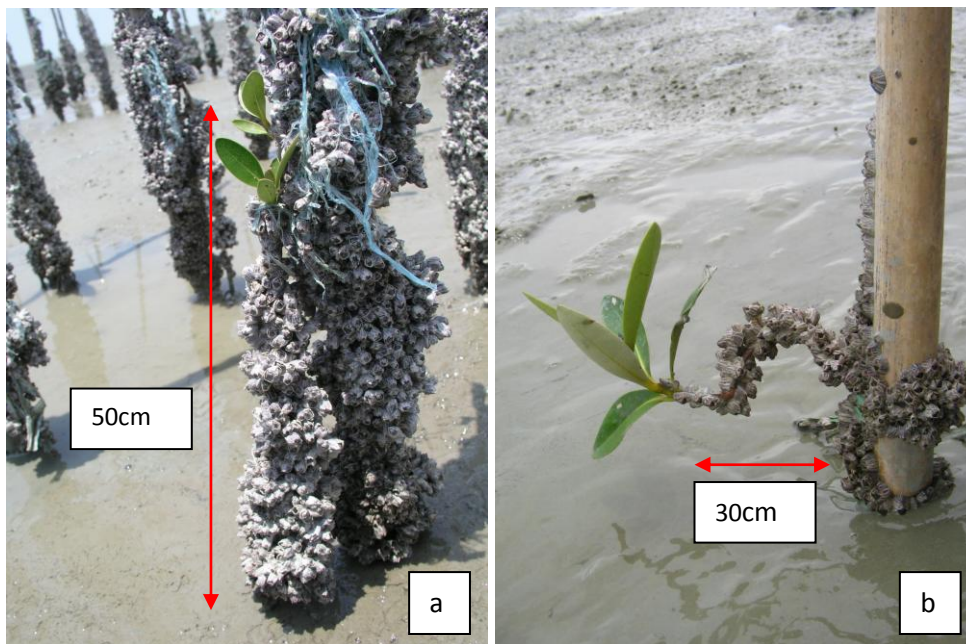
week	1	2	3	4	5	6	7	8	9
rate colonization (no./week)	0.1	0.2	0.8	0.45	0.66	0.12	0.34	0.01	0.46

## CHAPTER 4: EFFECTS OF BIOFOULING ON REPLANTED MANGROVE SEEDLINGS

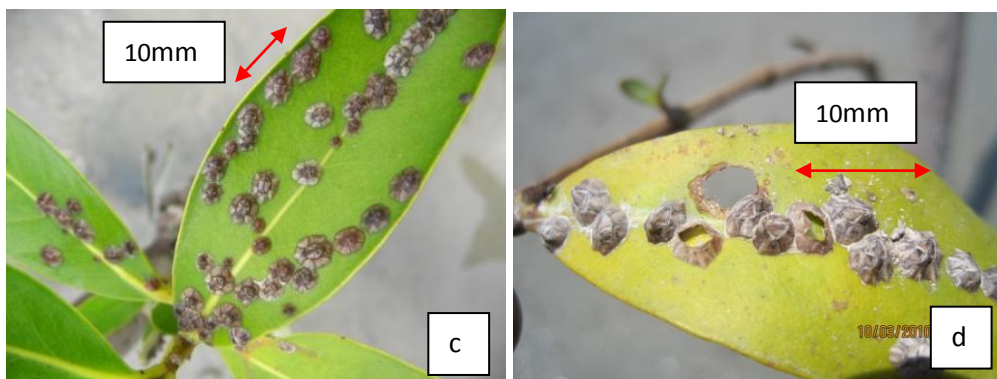
### 4.1. Physical damage caused by biofoulers.

In this study of one and half years (May 2008-October 2009), high infestation of barnacles on replanted seedlings was recorded (Experiment 1, 3, 4 and 5). Barnacles, for example *Amphibalanus amphitrite*, were the dominant biofouling organisms (more than 90%) on replanted mangrove seedlings (Figure 3.2a). The high density and coverage of barnacles (30% and above) obstructed the development of new leaves and shoots (Figure 4.1a). The weight load of barnacles could exceed six times the weight of the seedling. By the 7<sup>th</sup> week, the average weight of barnacles could reach up to 4.3g to 9.25g per plant (Experiment 1; Figure 4.4). Stems of the seedlings were unable to withstand such burden and the plants would bend and eventually collapse (Figure 4.1b). Barnacles also blocked the growth of roots from the stem (Figure 4.1a). The roots of *Rhizophora apiculata* are important because they are the support system and any blockage of root growth would weaken the support system. Barnacles were found attached on to the seedlings' leaves, stems and roots. Barnacles growth on the leaf surface (Figure 4.1c; Figure 4.1d) presumably reduces photosynthesis. However the physiological aspect of biofouling was not carried out in this study. Attachment of barnacles on the stem nodes of seedlings may hinder new leaves from sprouting (Figure 4.1e; 4.1f). Presumably, barnacles will reduce the photosynthetic capability of the plants. Barnacles would cause damage to the leaves if larvae settles on any tear or hole on the leaves (Figure 4.1g; 4.1h). For example, growth of barnacles on leaf hole will eventually cause the leaf to tear. The hard and sharp plates of adult barnacles may damage leaves or stem of seedlings if the wind or water current is strong (Figure 4.1i; 4.1j).

Gastropods (*Littoraria* sp) found on replanted mangrove seedlings were *Littoraria scabra* and *Littoraria melanostoma* (Figure 4.1k; 4.1l). These snails could be found under the leaves, stems and roots of the seedlings. *Littoraria scabra* were always found together in groups; however *Littoraria melanostoma* found solitary. The effects of gastropods on replanted seedlings were not obvious. *Limnoperna mangle* (mussels) were found growing among barnacles (Figure 4.1m). Mussels may increased the burden on young mangrove seedlings and prevent new leaves and roots from sprouting.



**Figure 4.1(a)** Barnacles had cover of more than 90% on replanted mangrove seedlings. The roots were unable to grow and seedlings appeared unhealthy. **(b)** A young mangrove plant weighed down by its burden of barnacles. The load weight of barnacles could exceed six times the weight of the plant (Figure 4.4).

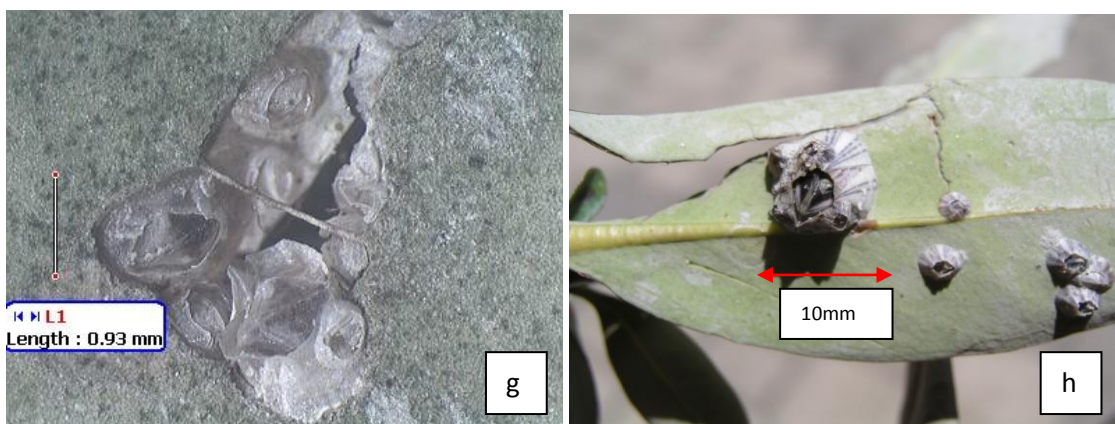


**Figure 4.1(c) and (d)** *Euraphia withersi* was found mostly on leaves. High coverage of *Euraphia withersi* on leaves might reduce rate of photosynthesis.

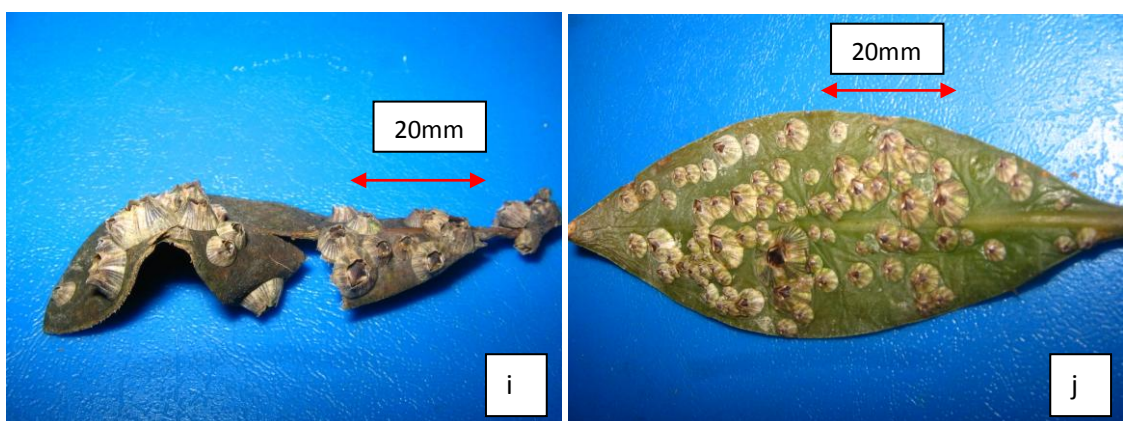




**Figure 4.1(e)** Before barnacle removal. Barnacles blocked the growth of new leaves.  
**(f)** After barnacle removal.

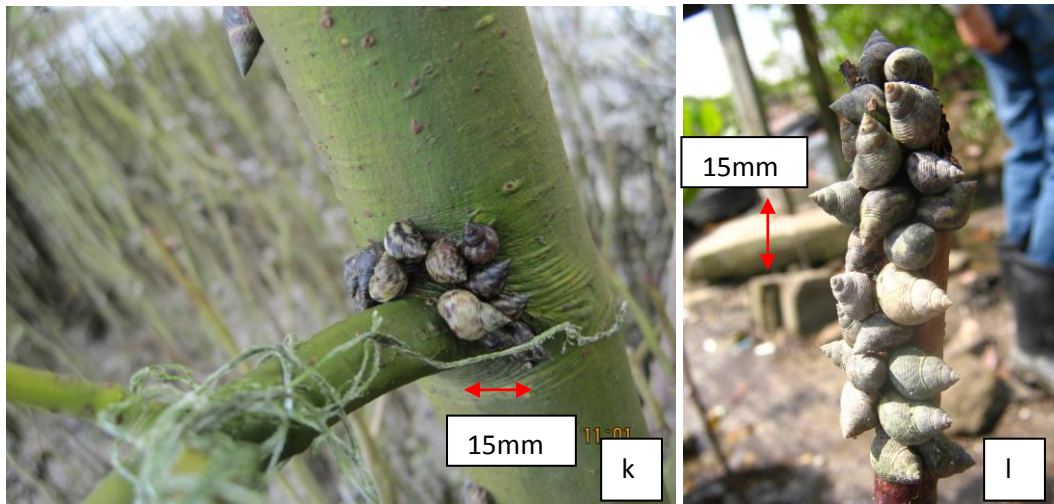


**Figure 4.1(g) and (h)** Growth of barnacles caused direct leaf damage if the larvae settled on the edge of small lacerations or pin holes on the leaf which resulted in leaf tearing.

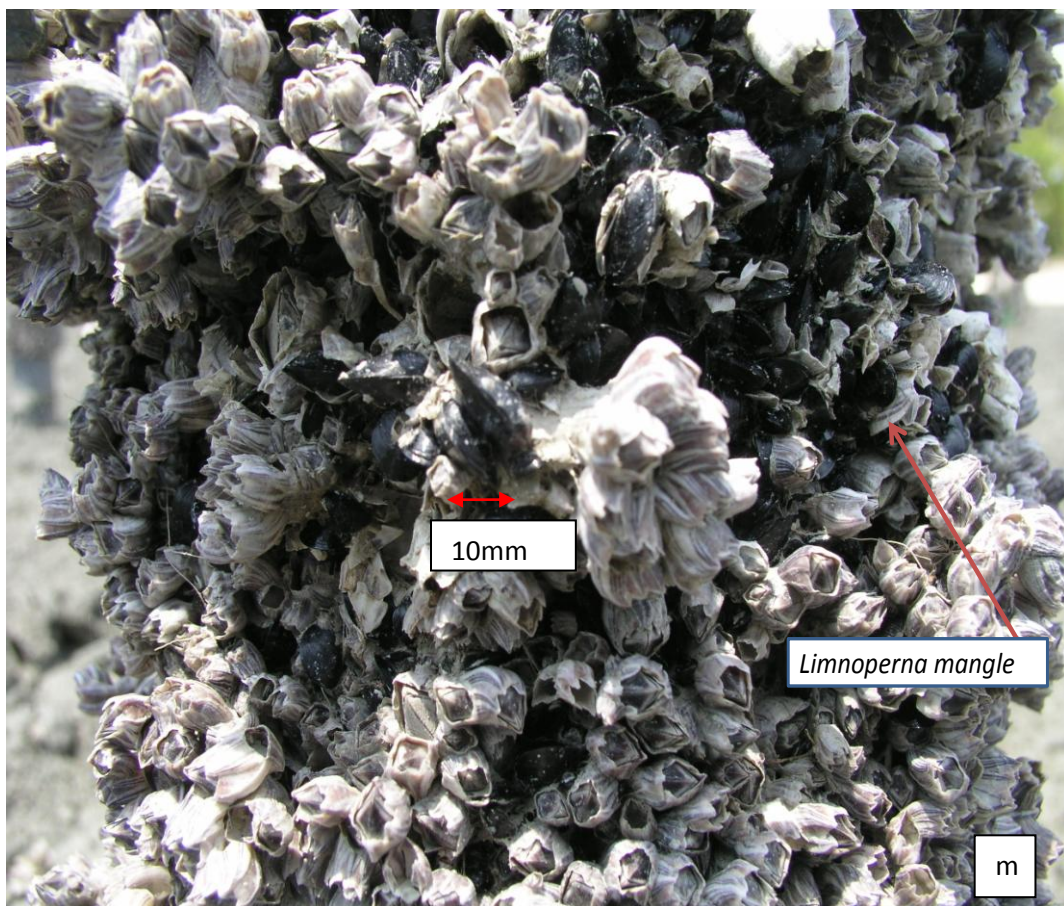


**Figure 4.1(i).** Hard and sharp plates of attached adult barnacles on swaying leaves or stem could cut and damage the plant. **(j)** High density of barnacles on leaves could reduce the rate of photosynthesis.





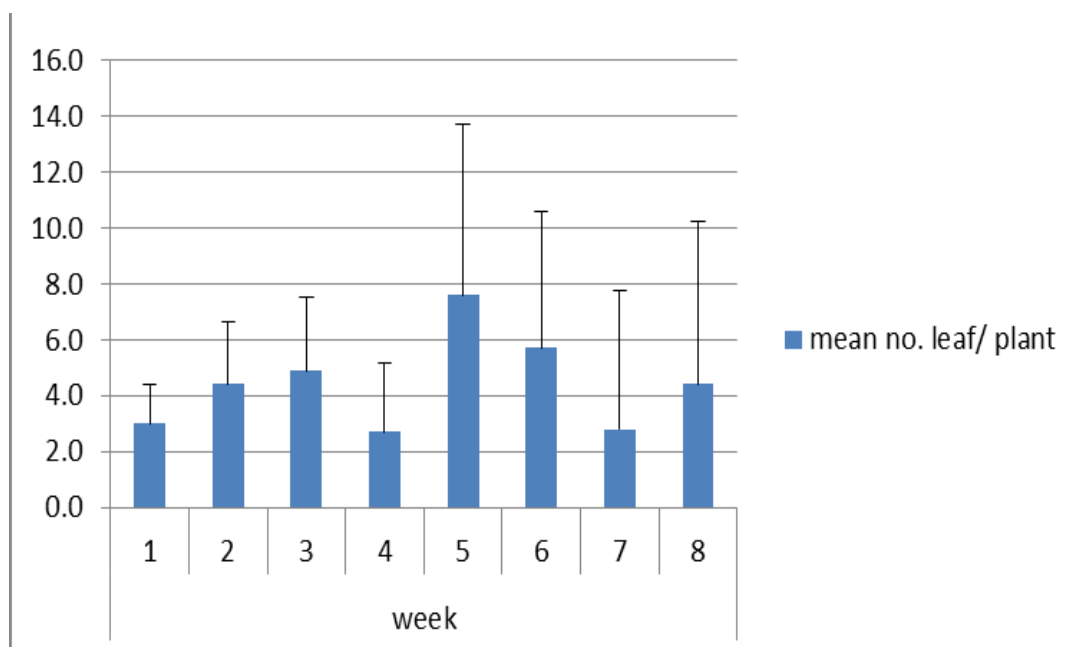
**Figure 4.1(k) and (l)** *Littoraria scabra* may increase the burden on young plants stem.



**Figure 4.1(m)** Competition for space by *Limnoperla mangle* and barnacles on the seedling stem. The biofoulers increased the load burden on the young mangrove seedling.

## 4.2 Number of *Avicennia marina* leaves during barnacles infestation over a period of 8 weeks (Experiment 1)

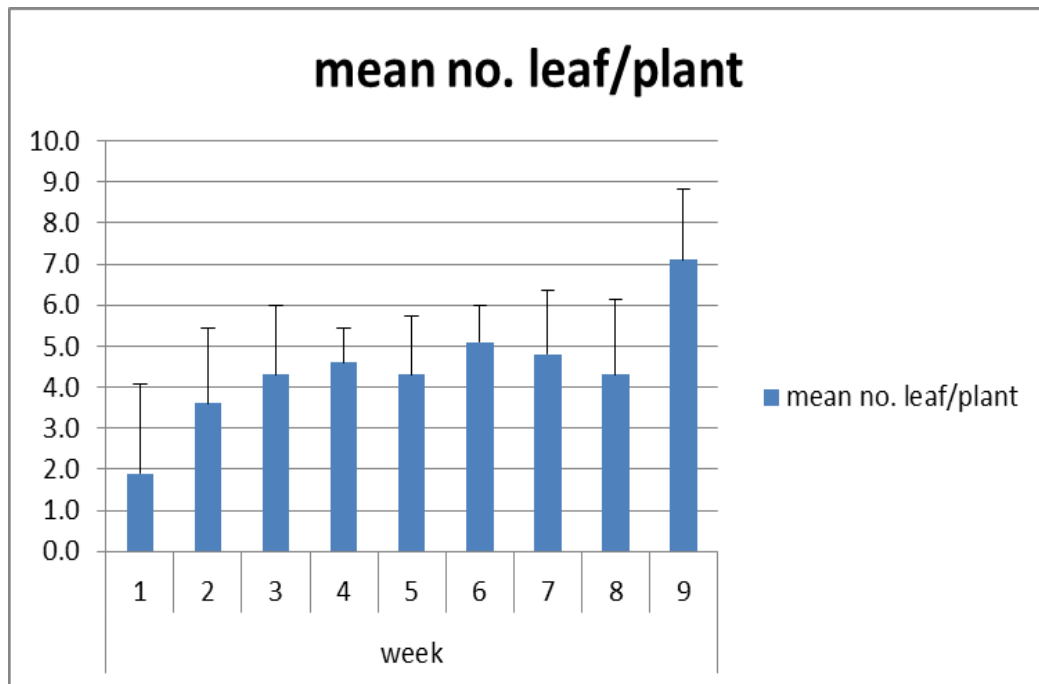
The mean number of mangrove leaves varied but was not significantly different over the period of eight weeks (Figure 4.2; Appendix 3a).



**Figure 4.2** Mean number of *Avicennia marina* leaves in a period of 8 weeks. Vertical bars denote standard deviation.

## 4.3 Number of *Rhizophora apiculata* leaves during barnacles infestation over a period of 9 weeks (Experiment 2)

The mean number of mangrove leaves increased significantly over a period of nine weeks ( $p < 0.05$ ; Appendix 3b). The number of *Rhizophora apiculata* leaves at the 1<sup>st</sup> week (1.9 no./plant) was significantly lower compared to the others, except at the 2<sup>nd</sup> week (3.6 no./plant ;  $p < 0.05$ ; Figure 4.3; Appendix 3c). The number of leaves at the 9<sup>th</sup> week (7.2no./plant) was significantly higher compared to others except in the 6<sup>th</sup> week (5 no./plant;  $p < 0.05$ ).



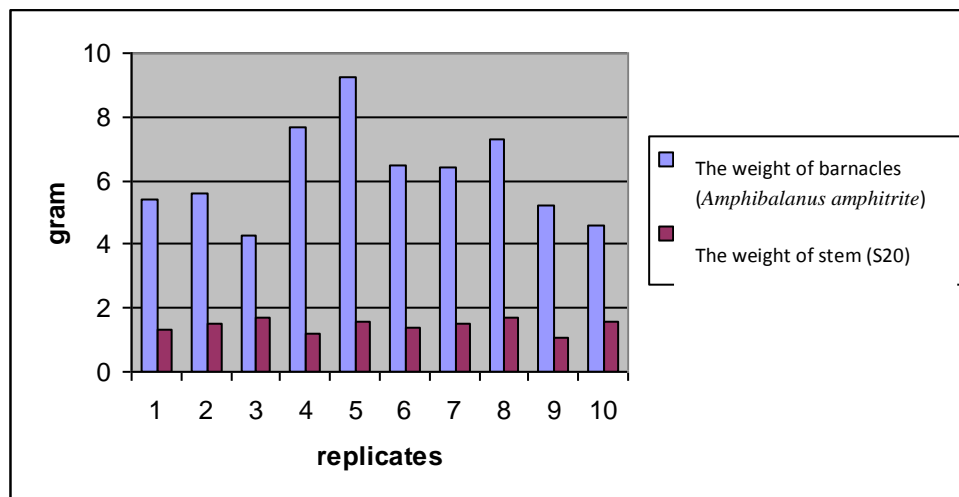
**Figure 4.3** Mean number of *Rhizophora apiculata* leaves during barnacles infestation over a period of 9 weeks. Vertical bars denote standard deviation.

#### **4.4 Shell diameter of barnacles on *Avicennia marina* seedlings. (Experiment 1)**

The mean shell diameter of barnacles on *Avicennia marina* seedlings increased from week 1 ( $0.78\text{mm} \pm 0.27$ ) until week 7 ( $4.48\text{mm} \pm 0.75$ ; Appendix 3d). The mean growth rate of barnacles was  $0.53\text{mm/week}$ .

#### **4.5 The total weight of the barnacles on *Avicennia marina* seedlings at week 7.**

At week 7, the total weight of barnacles (*Amphibalanus amphitrite*) reached 4.3g to 9.25g per 10cm stem length (S20) (Figure 4.4). The weight load of the barnacles could exceed six times the weight of the host seedling. High density of barnacles may create a physical drag and weight loading on the plant.



**Figure 4.4** The mean weight of total barnacles (*Amphibalanus amphitrite*) as compared to weight of *Avicennia marina* seedlings stem at section S20 (stem10-20cm height above ground) at week 7.

#### **4.6. Effects of removal of biofouling organisms on survival of *Avicennia marina*, *Rhizophora mucronata* and *Rhizophora apiculata* seedlings (Experiment 3).**

For *Avicennia marina*, the percentage of biofouling organisms covering the seedlings in the “control+” group at the final week was  $11.8 \pm 6$  % (Table 4.1). The effect of the treatments on the number of leaves and seedling survival rate was not significantly different ( $p > 0.05$ ; Appendix 3e).

For *Rhizophora mucronata*, the percentage coverage of biofouling organisms on the seedlings in the control+ group at the final week was  $19 \pm 4$ %. The effect of the treatments on the number of leaves was not significantly different ( $p > 0.05$ ; Appendix 3f). In all treatments, all the *Rhizophora mucronata* seedlings survived after 20 weeks.

For *Rhizophora apiculata*, the percentage coverage of biofouling organisms on the seedlings in the control+ group at the final week was 17±3%. The effect of treatments on the number of leaves and seedling survival rate was not significantly different ( $p>0.05$ ; Appendix 3g-3h)

**Table 4.1** Mean number of seedlings leaves and mean survival rate at the 20<sup>th</sup> week based on different treatments. (*Avicennia marina* seedlings were planted on Coir-log; C.D 2.5m; *Rhizophora mucronata* seedlings were planted using Comp-mat; C.D 2.5m and *Rhizophora apiculata* seedlings were planted on Coir-log; C.D 2.5m). Leaf =removal of biofouling organisms from the leaves, Stem= removal of biofouling organisms from the stem, Control - = removal of biofouling organisms from both leaves and stem, Control += Non-removal of biofouling organisms. SD denotes standard deviation.

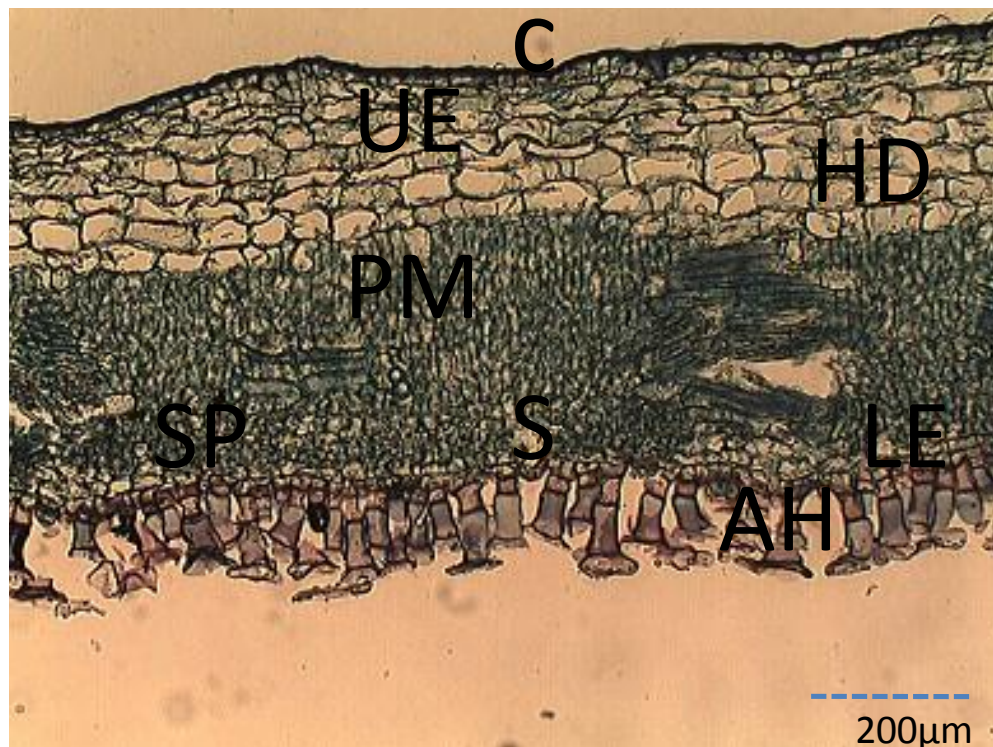
Treatment	<i>Avicennia marina</i> (n=20 )				<i>Rhizophora mucronata</i> (n=18 )				<i>Rhizophora apiculata</i> (n=15 )			
	Survival%		No. of leaves		Survival %		No. of leaves		Survival %		No. of leaves	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
Leaf	98.6	3.8	3.3	7.1	100	0	17.8	12.2	100	0	12.0	7.0
Stem	97.1	7.6	15.8	9.1	100	0	14.8	8.5	90	58.2	10.7	6.4
Control-	67.1	40.7	2.0	6.1	100	0	11.2	6.2	100	0	7.3	12.4
Control+	91.4	1	7.8	17.2	100	0	20.0	6.4	100	0	13.3	12.8

## 4.7 Effect of biofouling on plant tissues

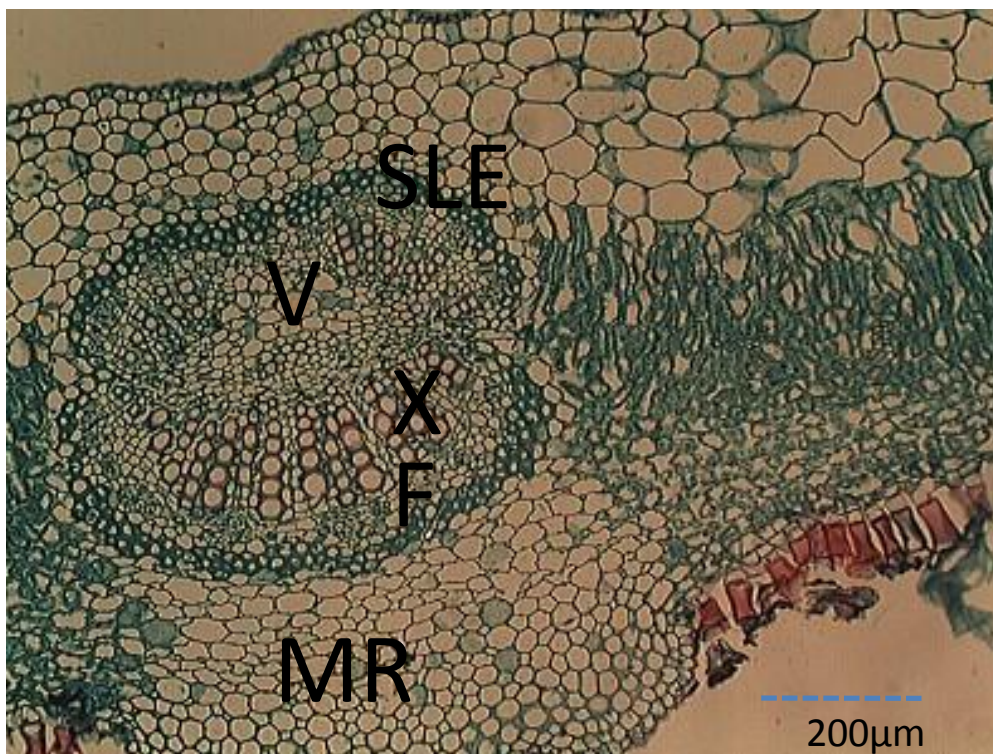
### 4.7.1 Histological studies of non-fouled *Avicennia marina* leaves (Control)

The *Avicennia marina* leaf lamina is ovate; the upper surface is yellow green in colour. The lower surface is covered with short abaxial hair. The leaf has a thick layer of hypodermis and palisade mesophyll. The phloem and xylem tissues are found on primary veins (Figure 4.5 and 4.6).





**Figure 4.5** Cross-section of non-fouled *Avicennia marina* leaf. (C) Cuticle, (UE) Upper epidermis, (HD) Hypodermis, (PM) Palisade mesophyll, (SP) Spongy Palisade, (S) Stoma, (AH) Abaxial hair.

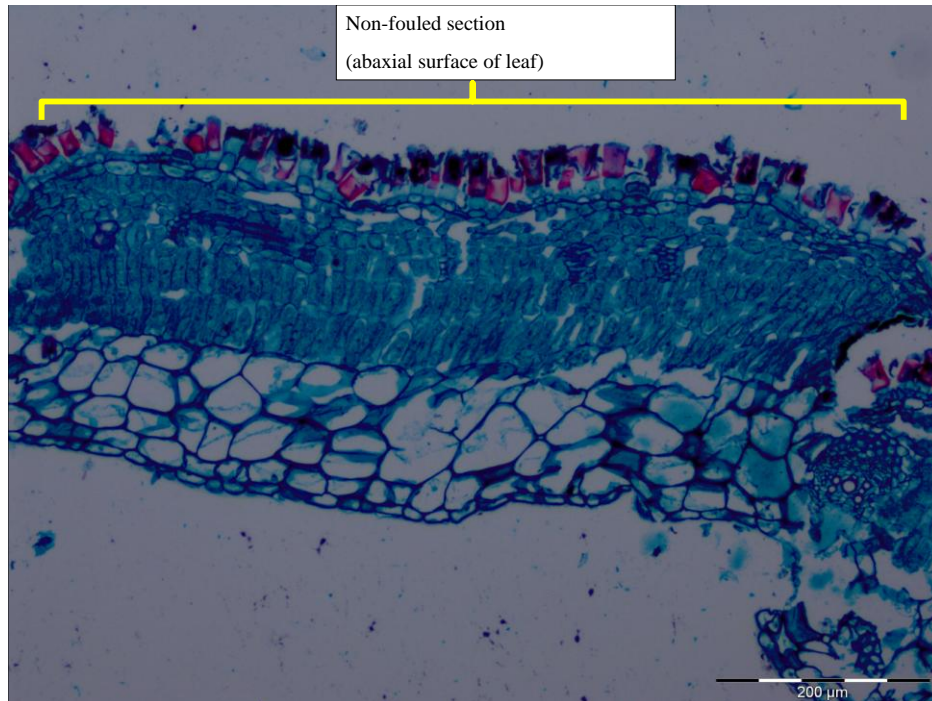


**Figure 4.6** Cross-section of non-fouled *Avicennia marina* leaf across mid rib (MR, left) and leaf blade, showing xylem and phloem tissue. (SLE) Sclerenchyma cells, (V) Vessel, (X) Xylem, (F) Phloem, (MR) Mid rib.

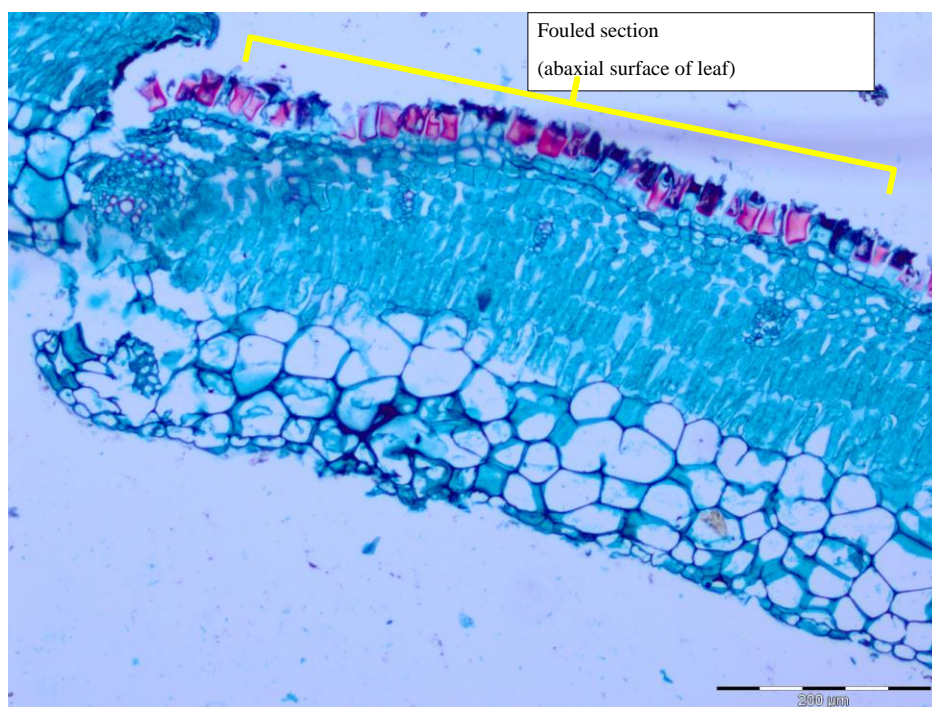


#### 4.7.2 Comparison of fouled and non-fouled sections to show barnacle attachment effect on abaxial surface of *Avicennia marina* leaf.

The fouled and non- fouled sections on the abaxial surface of *Avicennia marina* leaf did not differ histologically in term of structure of their abaxial hair and mesodermal layer (Figure 4.7a; Figure 4.7b).



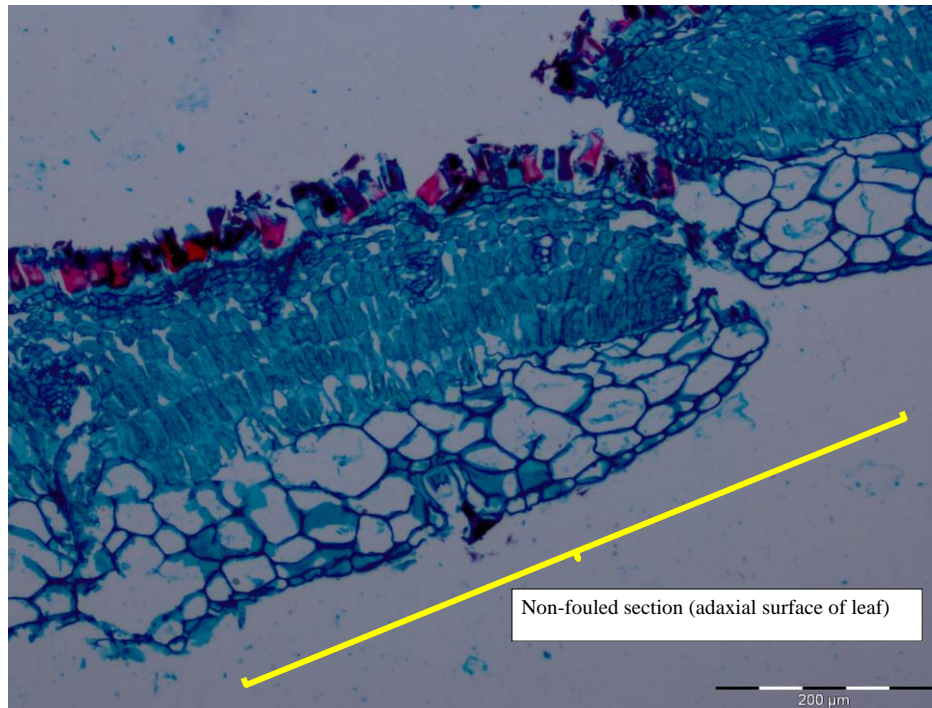
**Figure 4.7a** Enlarged view of non- fouled section (abaxial surface of leaf).



**Figure 4.7b** Enlarged view of fouled section (abaxial surface of leaf).

#### 4.7.3 Comparison of fouled and non- fouled sections to show barnacles attachment effect on adaxial surface of *Avicennia marina* leaf.

The fouled and non- fouled sections on the adaxial surface of *Avicennia marina* leaf did not differ histologically in term of structure of their abaxial hairs and mesodermal layer (4.8a, 4.8b).



**Figure 4.8a** Enlarged view of non- fouled section (adaxial surface of leaf).



**Figure 4.8b** Enlarged view of fouled section (adaxial surface of leaf).



#### 4.7.4 Effect of barnacles on percentage thickness of palisade layer and number of abaxial hairs of fouled and non-fouled leaves.

The results of paired-t test showed that the fouled leaf sections has significantly lower mean percentage thickness of palisade layer ( $53.2\% \pm 1.64$ ) compared to that of non-fouled leaves ( $56\% \pm 0.84$ ;  $p < 0.05$ ; Table 4.2; appendix 3i).

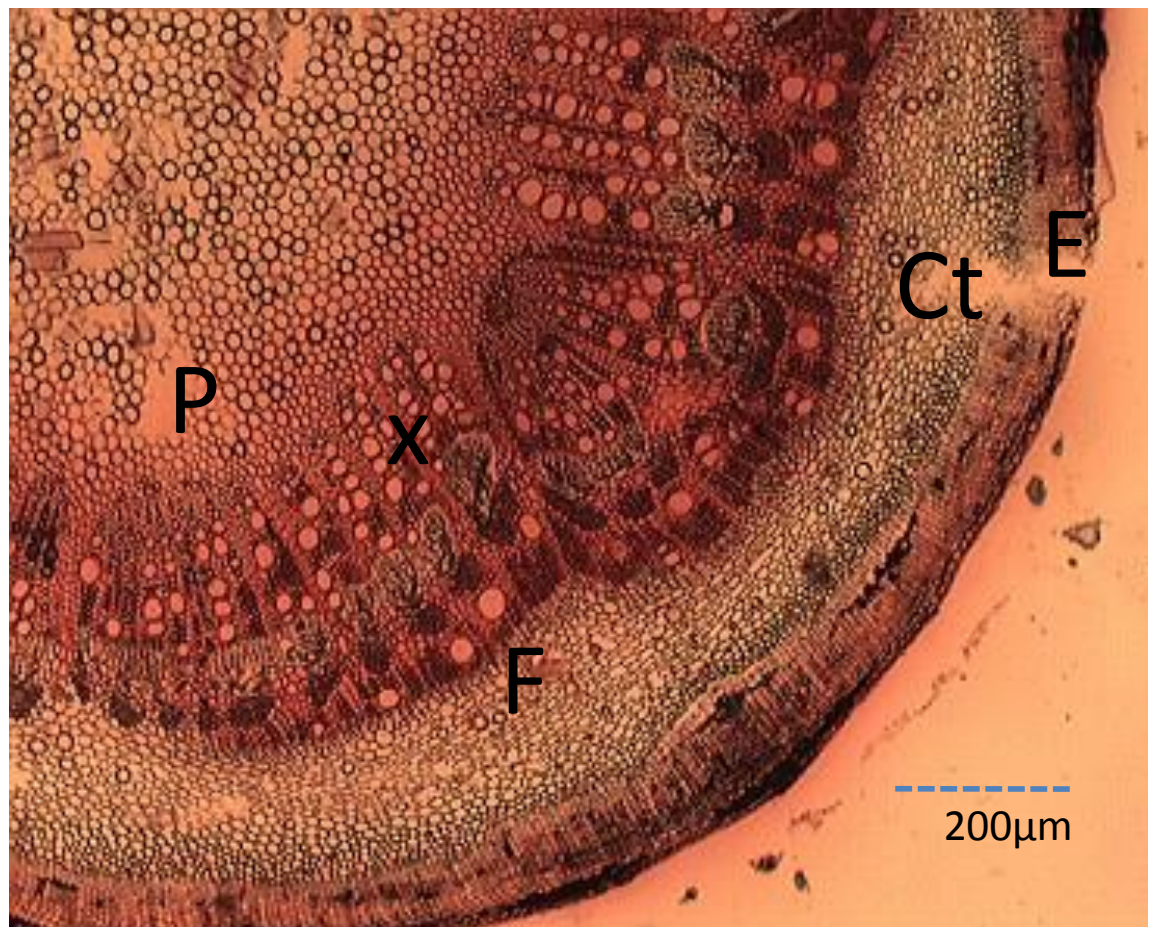
The fouled leaf sections has a higher mean number of abaxial hairs (7 no./500 $\mu$ m of lower epidermis) compared to leaves sections without fouling (5.6 no./ 500 $\mu$ m of lower epidermis). However, paired-t test showed that the effect of foulers on the number of abaxial hairs was not significantly different ( $p > 0.05$ ; Table 4.2).

**Table 4.2** Comparison of mean percentage thickness of palisade layer and number of abaxial hairs of leaves between non-fouled and fouled mangrove leaves

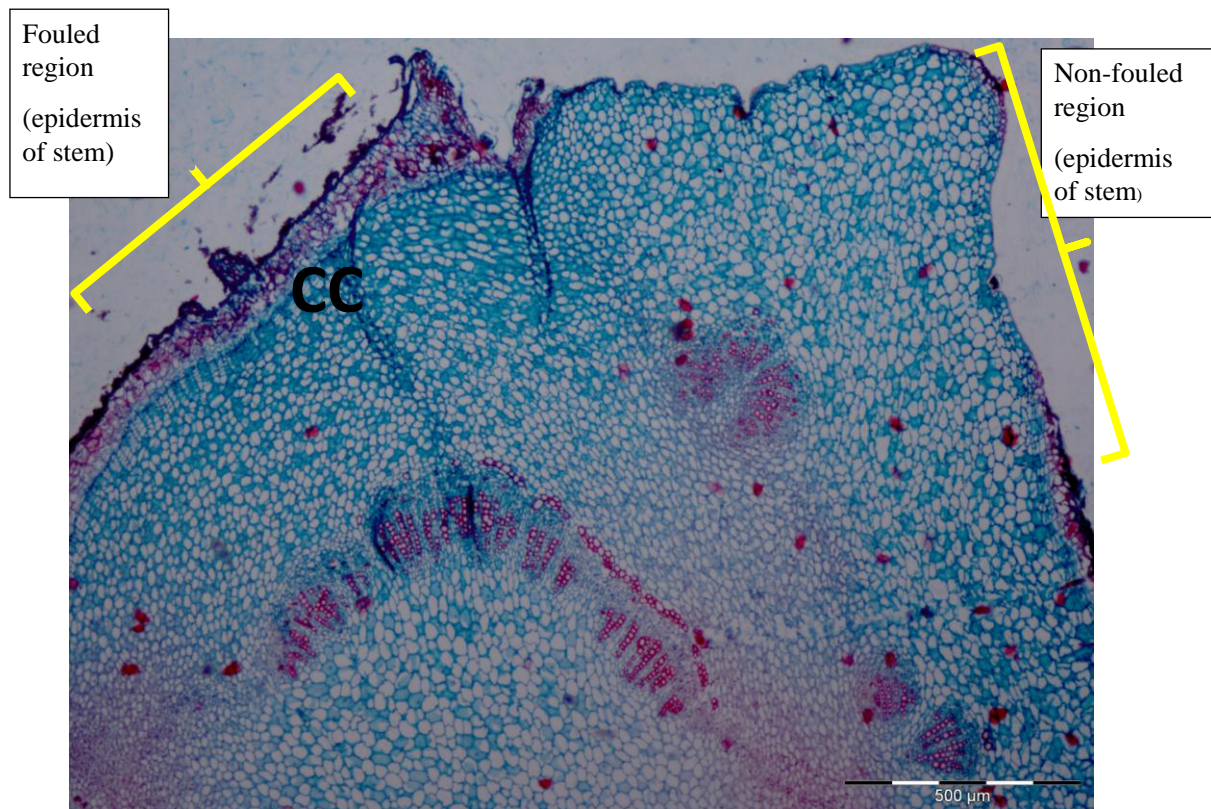
Characters	non-fouled surface of leaf(n=10)		fouled surface of leaf (n=10)	
	mean	SD	mean	SD
Palisade thickness (%)	56	0.84	53.2	1.64
No. of abaxial hairs (in 500 $\mu$ m layer of lower epidermis)	5.6	0.55	7.0	0.71

#### 4.7.5 Comparison of fouled and non- fouled sections to show barnacle attachment effect on stem of *Avicennia marina*.

The fouled sections on epidermis of *Avicennia marina* stem had significantly ( $p < 0.01$ ) thicker ( $146\mu\text{m} \pm 14.32$ ) and rapid growth of cork cambium as compared to non-fouled sections ( $72.4\mu\text{m} \pm 9.81$ ; Figure 4.10a; 4.10b). This may be the response of the seedlings to the attachment of barnacles



**Figure 4.9** Transverse section of non-fouled *Avicennia marina* stem (10-20cm height above ground). (E) Epidermis, (Ct) Cortex, (P) Pith, (X) Xylem, (F) Phloem.



**Figure 4.10** Transverse section of *Avicennia marina* stem (10-20cm height above ground) showing the development of cork cambium (CC) at the fouled region.

## CHAPTER 5: BIOFOULING CONTROL

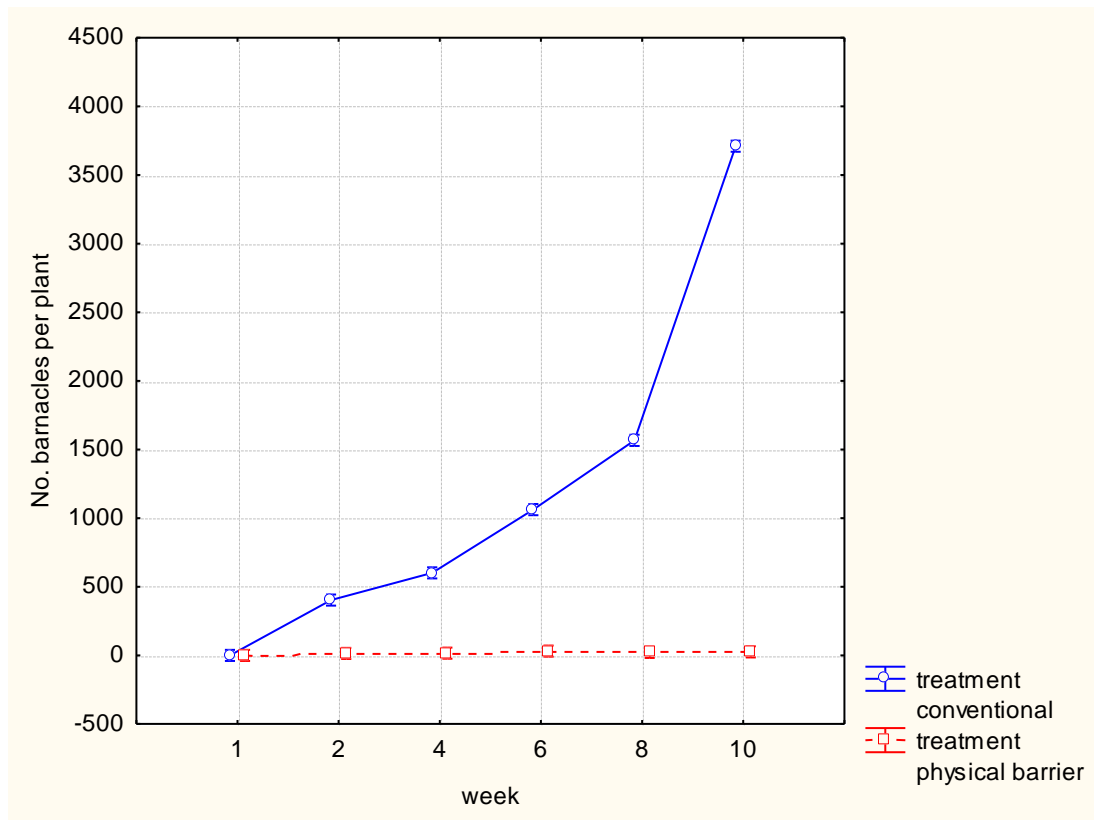
### 5.1 Study on using physical barrier to reduce biofouling organisms on replanted *Avicennia marina* seedlings (Experiment 4)

#### 5.1.1 Abundance of barnacles on seedlings planted using the conventional method and physical barrier method.

The effect of treatment and time on abundance of barnacles was significantly different ( $p < 0.05$ ; Appendix 4a). The mean abundance of barnacles found on seedlings using conventional method (1223 indiv./plant) was significantly higher compared to the physical barrier method over a period of 10 weeks (18 indiv./plant;  $p < 0.05$ ; Appendix 4b). These results supported the hypothesis that physical barrier method is able to reduce growth of biofouling organisms on mangrove seedlings.

#### 5.1.2 The interaction effect of time and treatments on the abundance of barnacles.

The interaction effect of time and treatments on the abundance of barnacles showed significant difference ( $p < 0.001$ ; Appendix 4a). The mean abundance of barnacles found on seedlings using the conventional method was significantly higher compared to the physical barrier method used from the 2<sup>nd</sup> week to the 10<sup>th</sup> week ( $p < 0.001$ ; Figure 5.1; Appendix 4c). The abundance of barnacles found on seedlings using the conventional method or no protection showed a significant increase from the 4<sup>th</sup> week (601 indiv./plant) until the 10<sup>th</sup> week (3710 no./plant). The mean abundance of barnacles found on the seedlings protected by the PVC piping showed no significant difference from 2<sup>nd</sup> week (14 indiv./plant) to the 10<sup>th</sup> week (25.6 indiv./plant).



**Figure 5.1** The mean abundance of barnacles (indiv./plant) found on the *Avicennia marina* seedlings using the conventional method and physical barrier method in a period of 10 weeks.

### 5.1.3. Number of leaves of *Avicennia marina* seedlings using conventional method and physical barrier method.

The effect of treatment and time on the number of leaves of the seedlings was significantly different ( $p < 0.05$ ; Appendix 4d). The number of leaves on the seedlings using the conventional method of planting (4.05 no./plant) was significantly lower compared to the physical barrier method (5.61 no./plant;  $p < 0.05$ ; Appendix 4e).

### 5.1.4 The interaction effect of time and treatments on the number of leaves of the seedlings.

There was no interactive effect of time and treatments on the number of leaves of the seedlings ( $p > 0.05$ ; Appendix 4d).

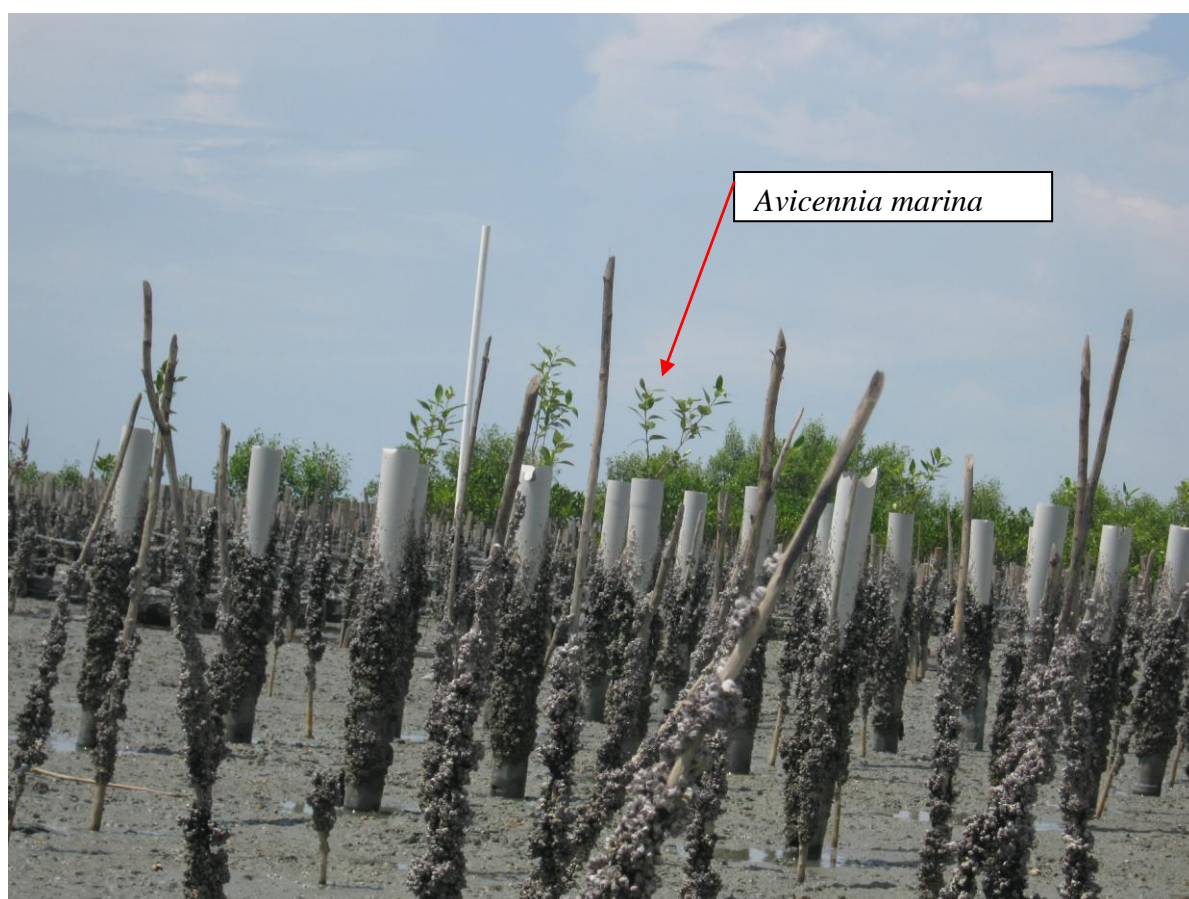


### 5.1.5 Health of seedlings based on leaf colour of planted seedlings using the conventional method and physical barrier method.

Colour of leaves using conventional method after 10 weeks was 33.33% yellow and 66.67% green. Colour of leaves using the physical barrier method after 10 weeks was 100% green.

### 5.1.6 Survival rate of seedlings using the conventional method and physical barrier method.

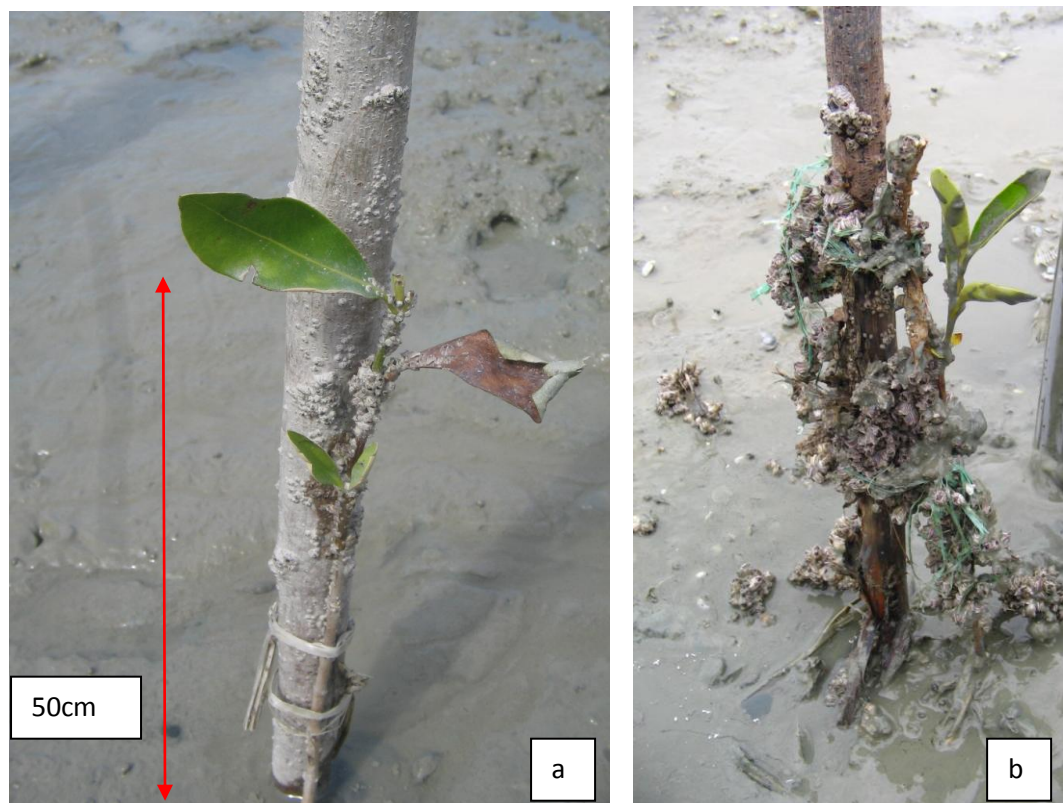
All seedlings planted using the conventional method died after 3 months. However, 50% of seedlings planted using the physical barrier method survived more than 2 years (Figure 5.2).



**Figure 5.2** Seedlings of *Avicennia marina* planted using the physical barrier method after 2 years. The PVC pipings had protected the mangrove seedlings.



**Figure 5.3 (a) and (b)** Seedling of *Avicennia marina* planted using physical barrier method. The seedling had grown more branches and new roots after 2 years.

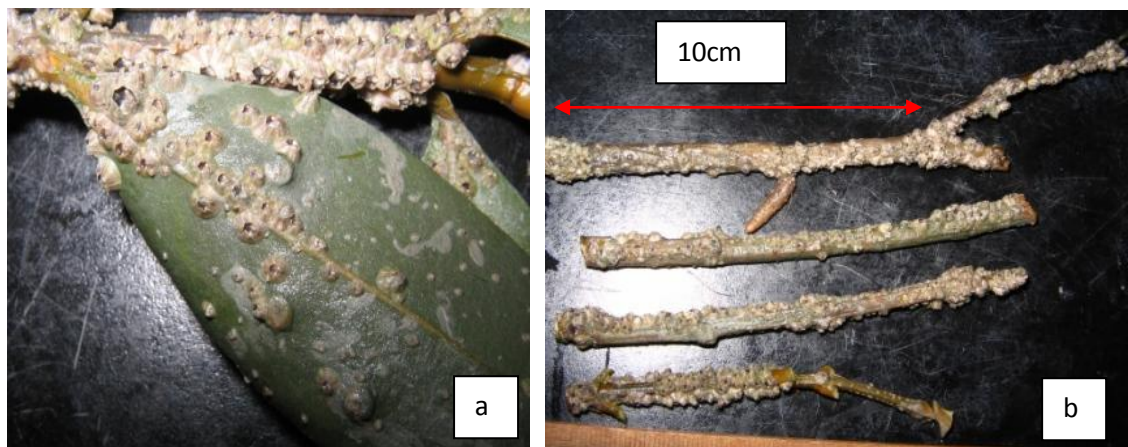


**Figure 5.4 (a) and (b)** Seedlings planted using the conventional method at the 3<sup>rd</sup> week. High density of barnacles had covered the mangrove seedlings.





**Figure 5.5 (a) and (b).** Seedlings planted using the physical barrier method at the 6<sup>rd</sup> week.



**Figure 5.6 (a) and (b)** Seedling planted using the conventional method at the 6<sup>th</sup> week. Barnacles grow on the all parts of the mangrove seedlings.

## 5.2 Study on effectiveness of the elevated and short stake method of planting to reduce biofouling on *Avicennia marina* seedlings (Experiment5)

### 5.2.1 The effect of treatment and block on abundance of barnacles

The effect of the treatment and block on the abundance of barnacles was significantly different ( $p < 0.05$ ; Appendix 5a). The mean abundance of barnacles on mangrove seedlings planted using the conventional method (21.3 no./plant) or control was significantly higher compared to the elevated and short stake methods ( $p < 0.001$ ), while



the mean abundance of barnacles on mangrove seedlings planted using the elevated method (1.5 no./plant) was significantly lower than the short stake method (5.5 no./plant;  $p < 0.001$ ; Figure 5.7; Appendix 5b). These results are supported by the hypothesis that less inundated mangrove seedlings will have less biofouling (Experiment 1, 2 and 3).

The mean abundance of barnacles at the block1 (9.79 no./plant) and block2 (11.74 no./plant) were no significantly different ( $p > 0.05$ ; Appendix 5c). However the mean abundance of barnacles at the block3 (4.54 no./plant) was significantly lower compared to block1 and block2 ( $p < 0.05$ ). This may because of block 3 was on the upper shore (2.5m above C.D) compared to block2 (2.0m above C.D) and block3 (1.6m above C.D) were the lower shore.

The interaction effect of block and treatment on abundance of barnacles showed significant difference ( $p < 0.05$ ; Figure 5.9; Appendix 5a). The mean abundance of barnacles planted using elevated method was no significant difference among each block ( $p > 0.05$ ; Appendix 5d). The mean abundance of barnacles planted using short stake method was no significantly different at the block 1 and 2, but significantly higher compared to block 3 ( $p < 0.05$ ). The mean abundance of barnacles planted using conventional method was no significantly different among each block ( $p > 0.05$ ).

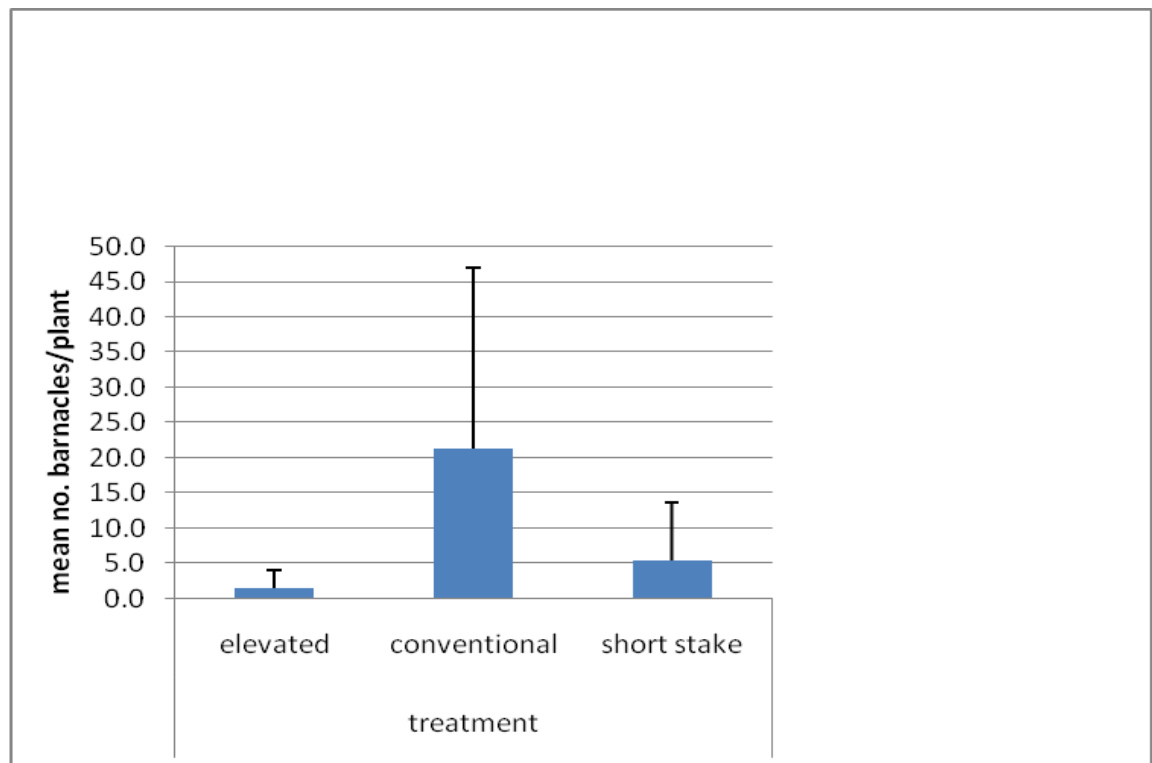
At the 1<sup>st</sup> week, the mean abundance of barnacles planted using conventional method (1.58 no./plant,  $SD \pm 2.53$ ) was significantly higher compared to the mean abundance of barnacles planted using elevated method (0.1 no./plant,  $SD \pm 0.3$ ) (Appendix 5e).

At the 3<sup>rd</sup> week, the mean abundance of barnacles planted using conventional method (5.34 no./plant,  $SD \pm 4.12$ ) was significantly higher compared to the mean abundance of barnacles planted using elevated method (0.27 no./plant,  $SD \pm 0.69$ ) and short stake method (1.76 no./plant,  $SD \pm 2.56$ ). However, the mean abundance of barnacles planted using elevated method was significantly lower compared to the mean abundance of barnacles planted using short stake method (Appendix 5f).

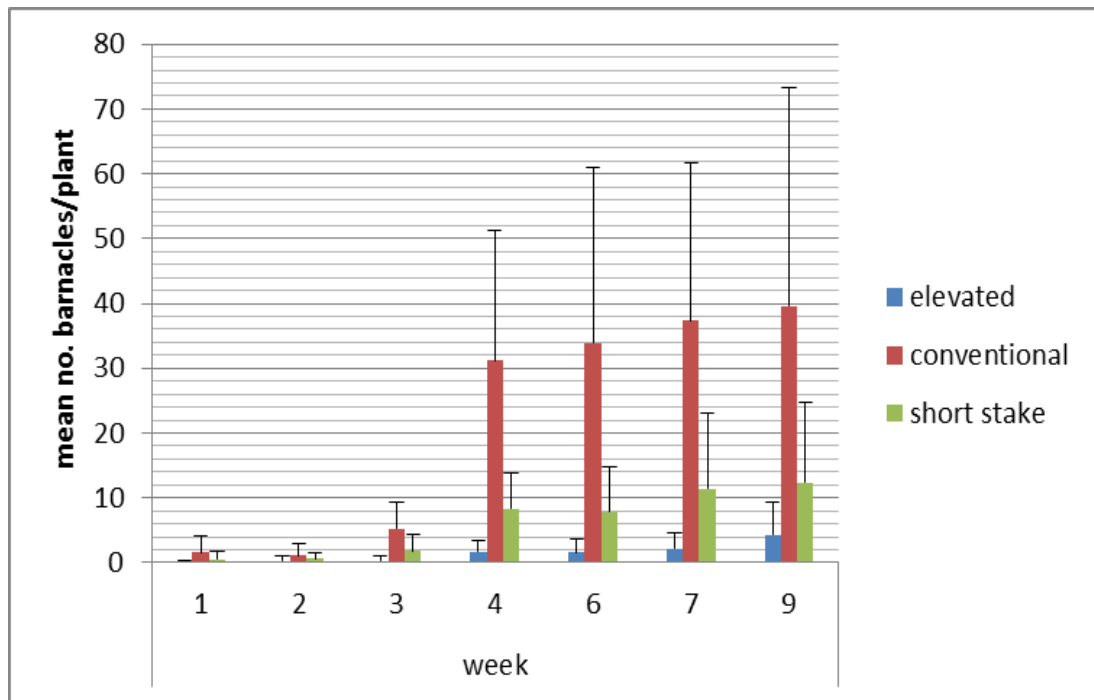
At the 6<sup>th</sup> week, the mean abundance of barnacles planted using conventional method (33.9 no./plant,  $SD \pm 27.1$ ) was significantly higher compared to the mean abundance of barnacles planted using elevated method (1.6 no./plant,  $SD \pm 2.1$ ) and short stake method (7.8 no./plant,  $SD \pm 7.1$ ). However, the mean abundance of barnacles planted using elevated method was significantly lower compared to the mean abundance of barnacles planted using short stake method (Appendix 5g).

At the 9<sup>th</sup> week, the mean abundance of barnacles planted using conventional method (33.59 no./plant,  $SD \pm 33.33$ ) was significantly higher compared to the mean abundance

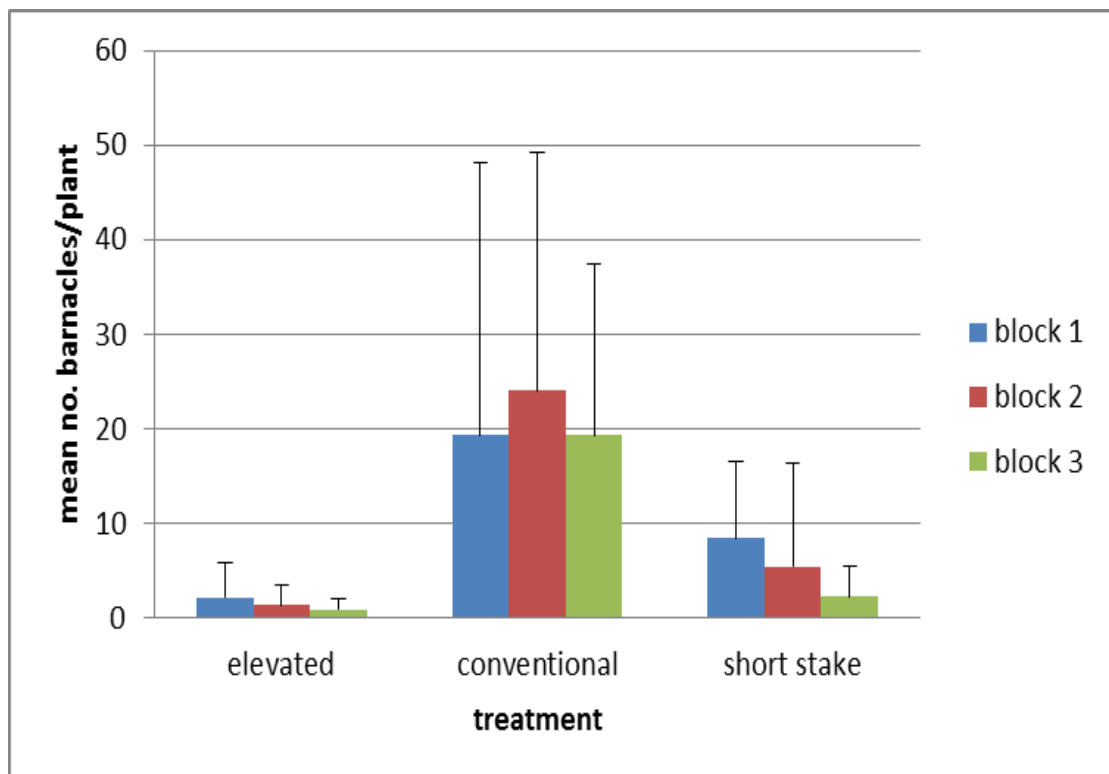
of barnacles planted using elevated method (4.3 no./plant,  $SD \pm 4.97$ ) and short stake method (12.4no./plant,  $SD \pm 12.41$ ). However, the mean abundance of barnacles planted using elevated method was no significantly difference compared to the mean abundance of barnacles planted using short stake method (Appendix 5h).



**Figure 5.7** The mean abundance of barnacles found on seedlings planted using three different planting methods. Vertical bars denote standard deviation.



**Figure 5.8** The mean abundance of barnacles found on seedlings planted using three methods for a period of 9 weeks. Vertical bars denote standard deviation.



**Figure 5.9** The mean abundance of barnacles found on seedlings planted using three methods at 3 blocks. Vertical bars denote standard deviation.

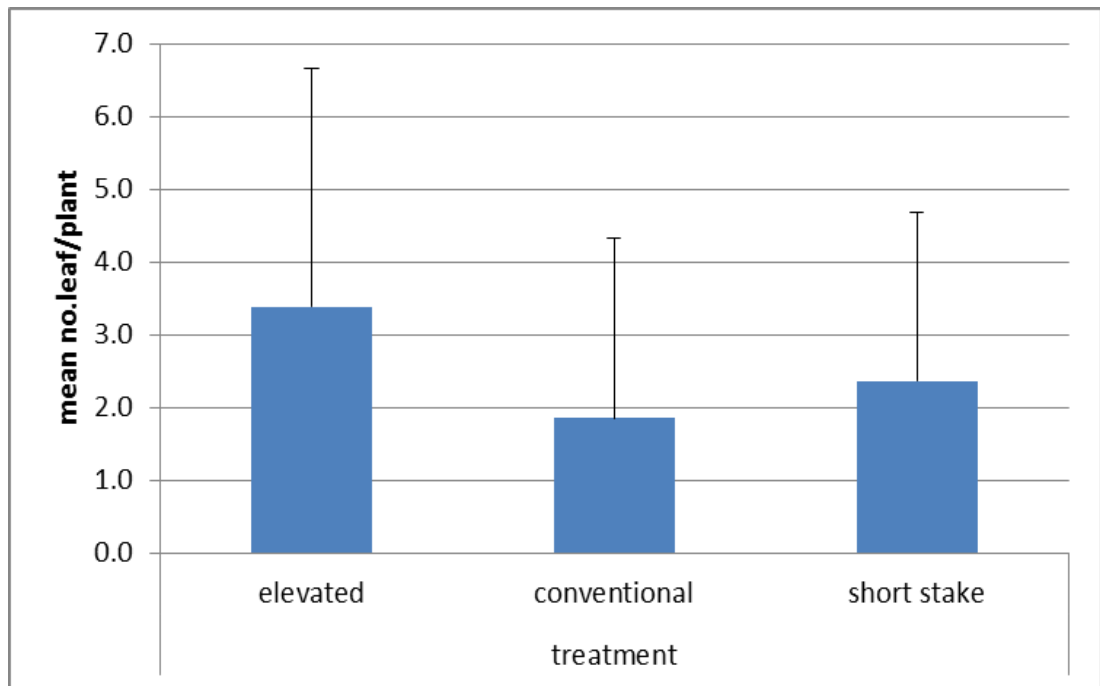
### **5.2.2 The effect of treatments on number of leaves.**

The effect of treatment on the mean number of leaves was significantly different ( $p < 0.001$ ; Appendix 5i). The mean number of leaves using the elevated method (3.4 no./plant) was significantly higher compared to the conventional and short stake methods ( $p < 0.001$ ; Figure 5.10; Appendix 5j). The mean number of leaves using the conventional method (1.9 no./plant) was no significantly different compared to the short stake method (2.4 no./plant;  $p > 0.05$ ).

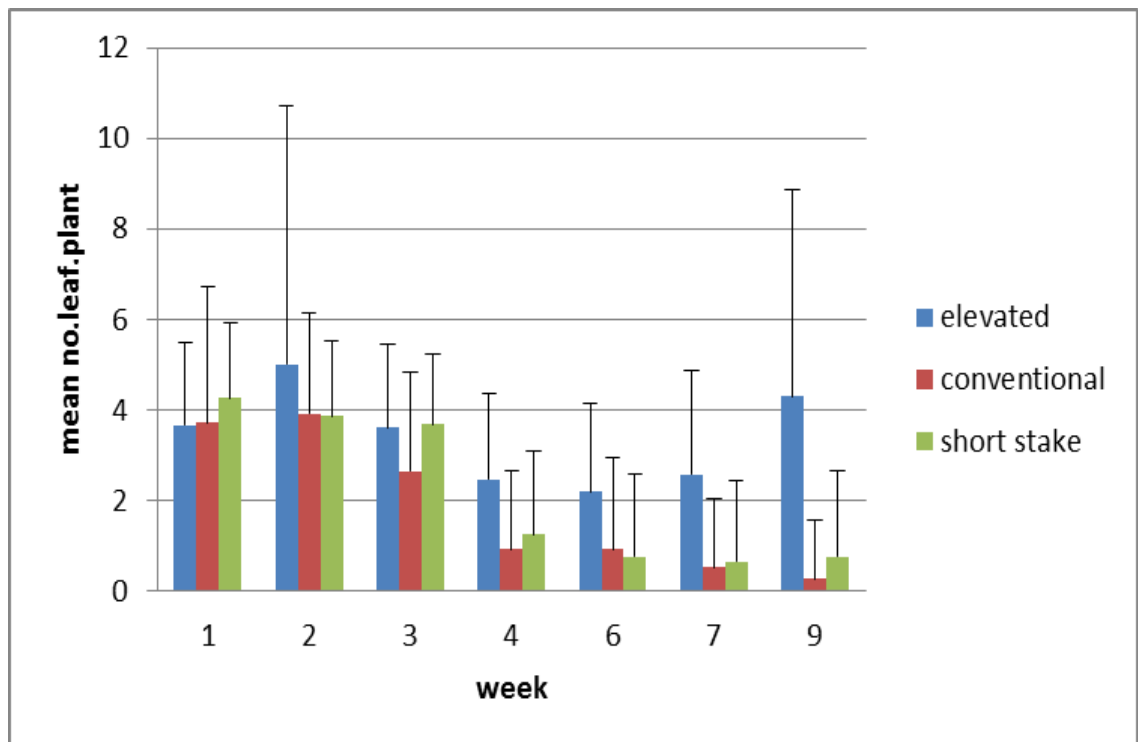
At the 1<sup>st</sup> and 3<sup>rd</sup> week, the mean number of leaves in all treatments were not significantly different ( $p > 0.05$ , Appendix 5k, 5l).

At the 6<sup>th</sup> week, the mean number of leaves in the plant planted using short stake method (0.75no./plant,  $SD \pm 1.85$ ) was significantly lower compared to the mean number of leaves in the plant planted using elevated method (2.2no./plant,  $SD \pm 1.96$ ) (Appendix 5n).

At the 9<sup>th</sup> week, the mean number of leaves in the plant planted using elevated method (4.3no./plant,  $SD \pm 4.6$ ) was significantly higher compared to the mean number of leaves in the plant planted using short stake method (0.75no./plant,  $SD \pm 1.92$ ) and conventional method (0.3 no./plant,  $SD \pm 1.3$ ) (Appendix 5o).



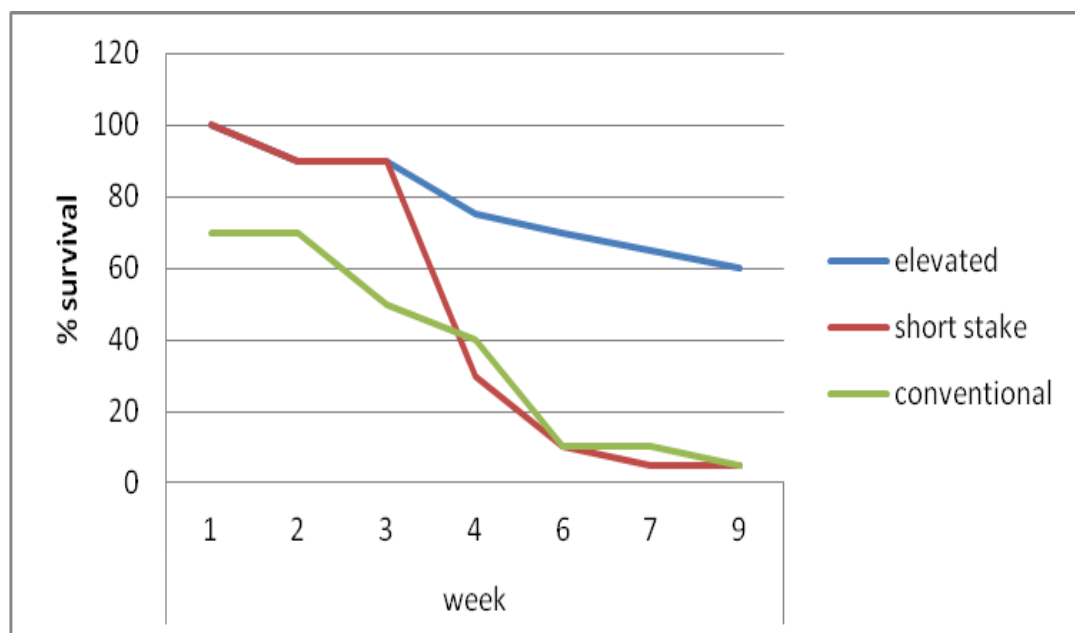
**Figure 5.10** The mean number of leaves using different planting methods. Vertical bars denote standard deviation.



**Figure 5.11** The number of leaves using different planting methods in period over 9 weeks. Vertical bars denote standard deviation.

### 5.2.3 Percentage survival of the seedlings.

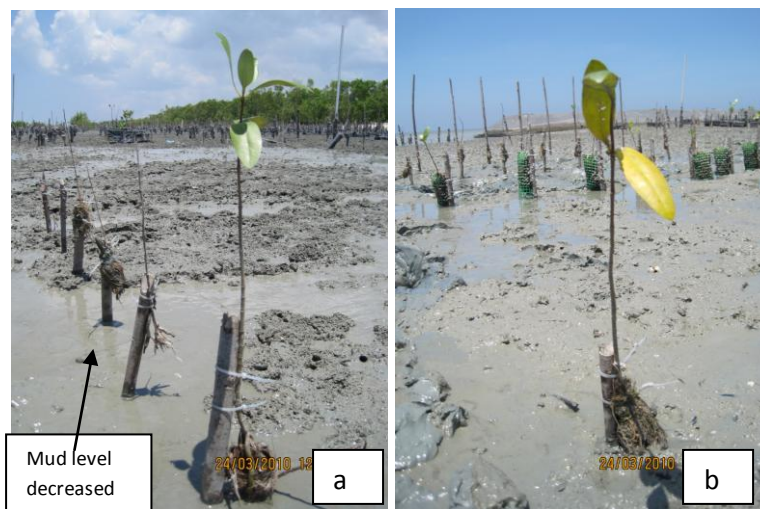
In the period after the 3<sup>rd</sup> week, the *Avicennia marina* seedlings planted using the elevated method had the highest percentage survival (60% at the 9<sup>th</sup> week) compared to the seedlings planted using the conventional method and short stake method (5% each at the 9<sup>th</sup> week; Figure 5.12). The percentage survival of seedlings using the conventional method and short stake method did not differ much.



**Figure 5.12** The percentage survival of the *Avicennia marina* seedlings using different planting methods in period of 9 weeks.



**Figure 5.13 (a) and (b)** Seedlings planted using the conventional method at 9<sup>th</sup> week.



**Figure 5.14 (a) and (b)** Seedlings planted using the short stake method at 9<sup>th</sup> week.



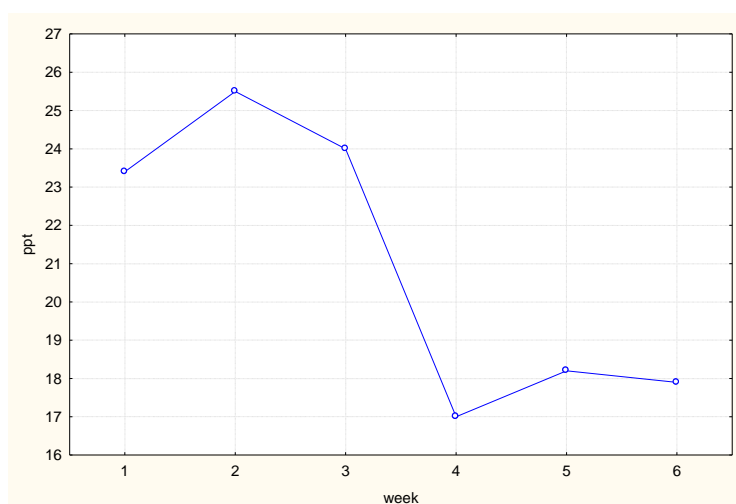
**Figure 5.15 (a) and (b)** Seedlings planted using the elevated method at 9<sup>th</sup> week.



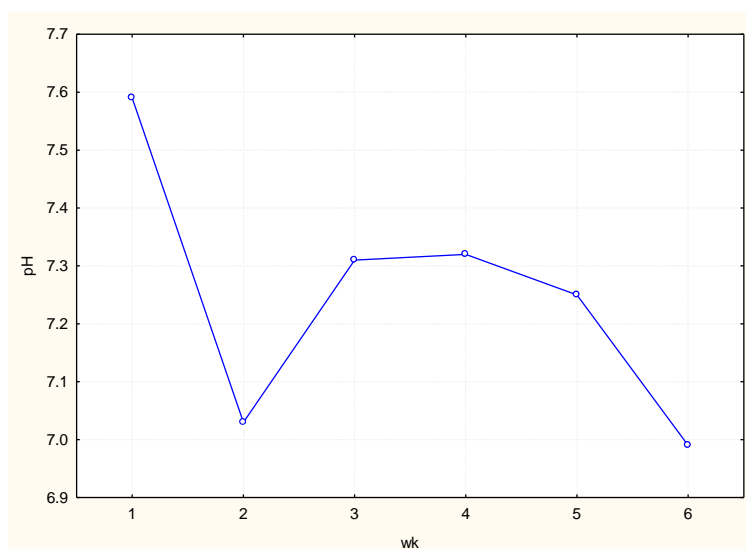
### 5.3 Effects of biocidal chemicals on fouling organisms and *Avicennia marina* seedlings (Experiment 6)

#### 5.3.1 Water parameters.

The range of the salinity of sediment water at KSL from early September 2009 until end of October 2009 (6 weeks) was 17ppt to 23.5ppt and the mean was 19 ppt. The range of the pH value of sediment water was 7 to 7.6 and the mean was 7.2 (Figure 5.16a, 5.16b).



**Figure 5.16a** The salinity (ppt) of the water in sediment at KSL from early September 2009 until end of October 2009.

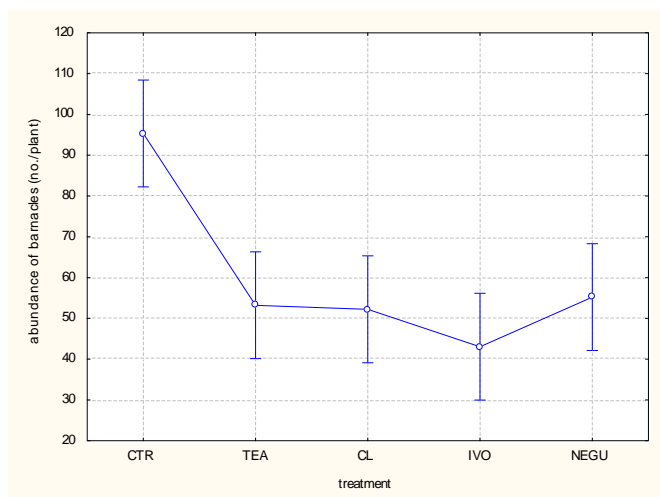


**Figure 5.16b** The pH value of the water in sediment at KSL from early September 2009 until end of October 2009.

### **5.3.2 Effect of treatment and time on abundance of barnacles**

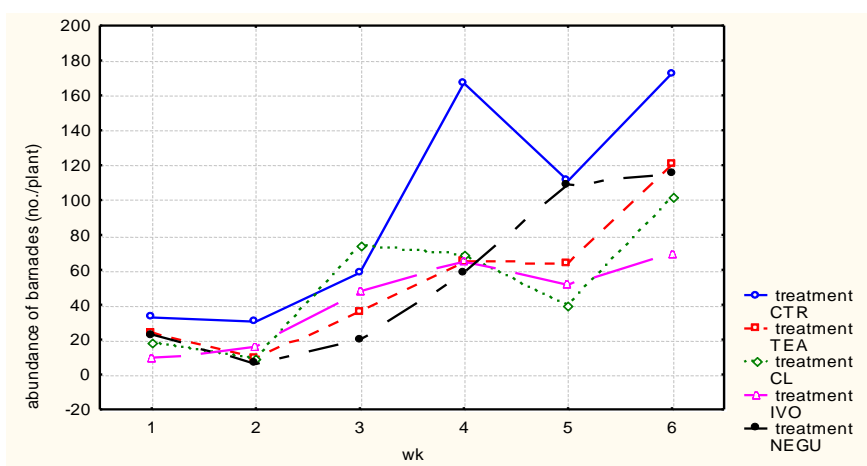
The effect of chemical treatment and time on the abundance of barnacles was significant (ANOVA;  $p < 0.001$ ; Appendix 6a). The mean abundance of barnacles in the control group (95.3 no./plant) was significantly higher compared to all treatment groups (Dunnett test,  $p < 0.05$ ; Figure 5.17; Appendix 6b). The mean abundance of barnacles treated using tea seed cake (53.2 no./plant), Clorox (52.2 no./plant), Ivermectin (43.1 no./plant) and Neguvon (55.2 no./plant) was not significantly different among each other (Tukey Test,  $p > 0.05$ , Appendix 6c). However, all these chemicals could not totally prevent biofouling. This may due to the removal or dilution of chemicals during high tide (inundation) or rain. For the field observation, the chemical used were not able to kill the adult barnacles. This may because of the chemicals were not able to pass through the operculum of the adult barnacles.

The mean abundance of barnacles at the 1<sup>st</sup> week (21.5 no./plant) was significantly higher compared to 2<sup>nd</sup> week (14.3 no./plant) ( $p < 0.05$ ; Appendix 6d). However, the mean abundance of barnacles at the 3<sup>rd</sup> week (47.3 no./plant) and 4<sup>th</sup> weeks (84 no./plant) were significantly increased ( $p < 0.05$ ). The mean abundance of barnacles from 4<sup>th</sup> week to 5<sup>th</sup> week (75 no./plant) were no significant difference ( $p > 0.05$ ). However the mean abundance of barnacles at the 6<sup>th</sup> week (116 no./plant) was significantly higher compared to 5<sup>th</sup> week ( $p < 0.05$ ).



**Figure 5.17** The effect of chemicals on mean abundance of barnacles from September 2009 until October 2009 (6 weeks) (CTR= control, TEA= tea seed, CL= Clorox, IVO = Ivermectin, NEGU= Neguvon). Vertical bars denote standard errors.

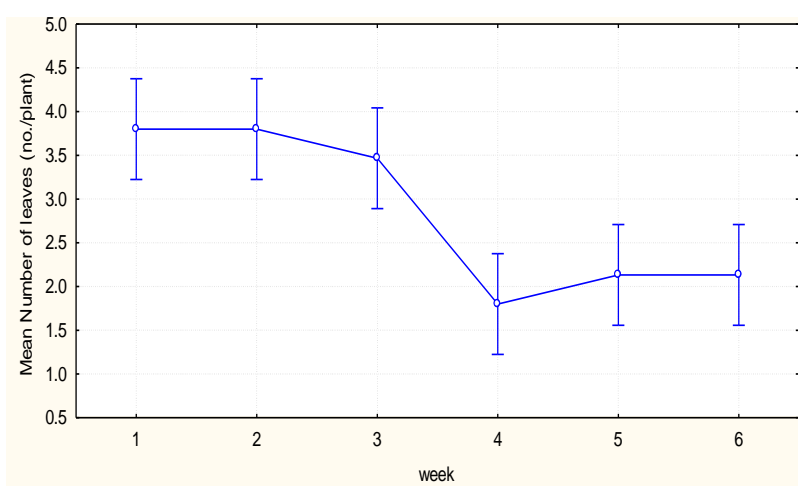
The interaction effect of time and treatment on the mean abundance of barnacles was significantly different ( $p < 0.05$ ; Figure 5.18; Appendix 6a). The mean abundance of barnacles among all chemical treatments and control showed no significant difference in the period between the 1<sup>st</sup> weeks until the 3<sup>rd</sup> week. However, the abundance of barnacles in the control group (167 no./plant) was significantly higher compared to the others at the 4<sup>th</sup> week ( $p < 0.05$ ; Appendix 6e). The abundance of barnacles on seedlings treated with Ivermectin and Clorox was significantly lower compared to the control group in the 4<sup>th</sup>, and 6<sup>th</sup> week ( $p < 0.05$ ).



**Figure 5.18** The mean abundance of barnacles found on seedlings treated using different chemicals in a period of 6 weeks. (CTR= control, TEA= tea seed, CL= Clorox, IVO = Ivermectin, NEGU= Neguvon).

### 5.3.3 Effect of biocidal chemicals on number of leaves

The effect of time on the mean number of leaves was significantly different ( $p < 0.05$ ; Appendix 6f). The number of leaves at the first three weeks was not significantly different ( $p > 0.05$ ; Figure 5.19; Appendix 6g). The mean number of leaves decreased significantly at the 4<sup>th</sup> week (1.8 no./plant) ( $p < 0.05$ ) compared to 3<sup>rd</sup> week (3.46 no./plant). The mean number of leaves showed no significant difference from 4<sup>th</sup> week until 6<sup>th</sup> week (2.13 no./plant). The effect of treatment and interaction effect of time and treatment on the mean number of leaves showed no significant difference ( $p > 0.05$ ; Appendix 6f).



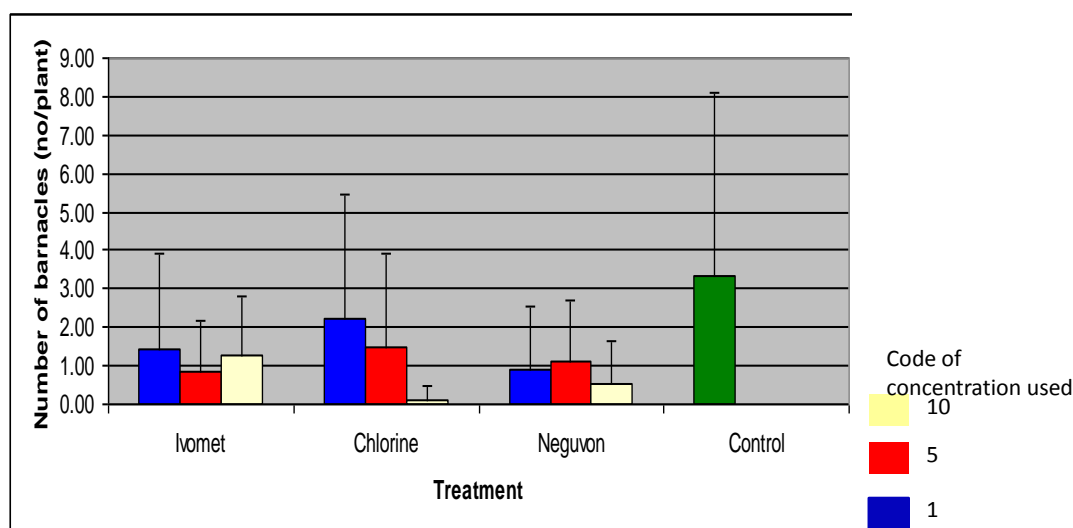
**Figure 5.19** The effect of time on the number of leaves of mangrove seedlings treated with biocides

## 5.4 Effects of different chemical concentrations on fouling of *Avicennia marina* seedlings (Experiment 7).

### 5.4.1 The effect of treatment of chemicals of different concentrations on abundance of barnacles on *Avicennia marina* seedlings

The effect of chemicals of different concentrations on the abundance of barnacles was significant (ANOVA;  $p < 0.001$ ; Appendix 7a). The mean abundance of barnacles in the

control group (3.4 no./plant $\pm$ 4.8; Figure 5.20) was significantly higher compared to all treatment groups with their concentrations (Dunnett test,  $p<0.05$ ; Appendix 7b). The mean abundances of barnacles treated with Ivermectin and Neguvon, for all their concentrations, were not significantly different among each other (Tukey Test,  $p>0.05$ ; Appendix 7c). The mean abundance of barnacles treated with Chlorine10 (0.13 no./plant $\pm$ 0.3) was significantly lower compared to barnacles treated with Chlorine1 and Chlorine5.

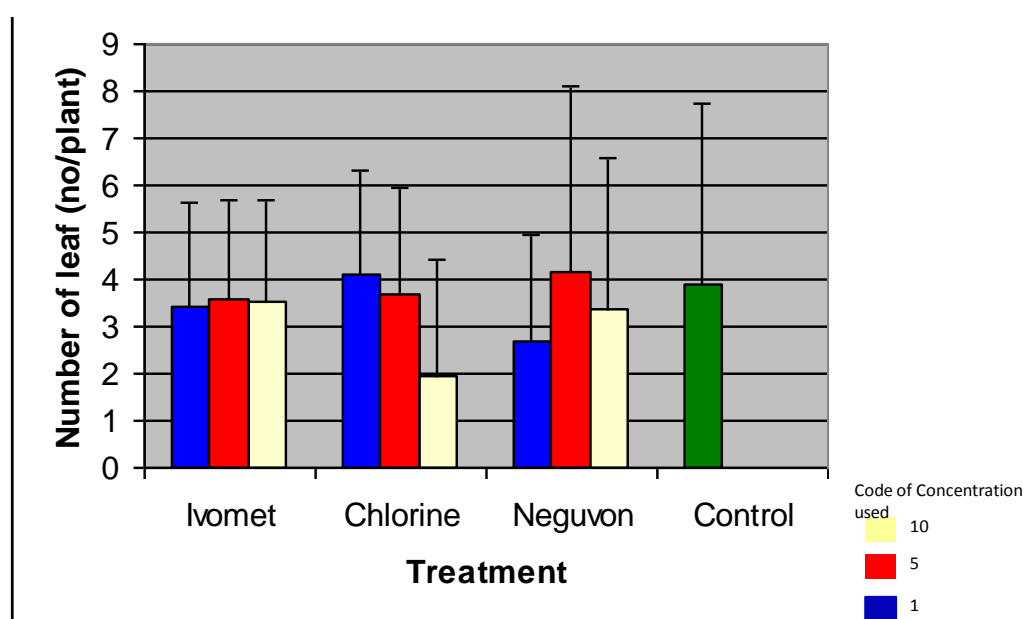


**Figure 5.20** The effects of different chemical concentrations on the abundance of barnacles on the seedlings. Ivermectin1=1 $\mu$ g /ml of Ivermectin, Ivermectin5=5 $\mu$ g /ml of Ivermectin, Ivermectin10=10 $\mu$ g /ml of Ivermectin, Chlorine1=0.5% chlorine, Chlorine5=2.5% chlorine, Chlorine10=5% chlorine, Neguvon1=0.97mg trichlorfon/ml, Neguvon5=4.85mg trichlorfon/ml, Neguvon10=9.7mg trichlorfon/ml, control=distilled water. Error bar showed standard deviation.

#### 5.4.2 The effect of treatment and concentration on number of leaves of *Avicennia marina* seedlings

The effect of treatment of chemicals of different concentrations was significantly affect the mean number of leaves ( $p<0.05$ , Appendix 7e). The mean number of leaves in plant

treated with all concentrations of Ivermectin ( $3.5 \text{ no./plant} \pm 2.1$ ) showed no significant different compared to that of the control group ( $p > 0.05$ ; Figure 5.21). This indicates Ivermectin did not disturb seedlings growth. Seedlings treated with Chlorine10 ( $1.94 \text{ no./plant} \pm 2.47$ ) had significantly lower number of leaves compared to the control group ( $3.88 \text{ no./plant}$ ;  $p < 0.05$ ). The number of leaves for the seedlings treated with Neguvon1 ( $2.71 \text{ no./plant} \pm 2.3$ ) was significantly lower compared to the control group ( $p < 0.05$ ).



**Figure 5.21** The effects of different chemical concentrations on the number of leaves . Ivermectin1=1 $\mu\text{g}$  /ml of Ivermectin, Ivermectin5=5 $\mu\text{g}$  /ml of Ivermectin, Ivermectin10=10 $\mu\text{g}$  /ml of Ivermectin, Chlorine1=0.5% chlorine, Chlorine5=2.5% chlorine, Chlorine10=5% chlorine, Neguvon1=0.97mg trichlorfon/ml, Neguvon5=4.85mg trichlorfon/ml, Neguvon10=9.7mg trichlorfon/ml, control=distilled water. Error bar showed standard deviation.

## 5.5 Effects of biocidal chemicals on beach fauna at study site (Experiment 8)

### 5.5.1 Animals found at study site

The animals counted visually in the quadrats were three species of crabs, 1 species of mud skipper, and three species of gastropods. The macrofauna collected by sieving with 500 $\mu\text{m}$  test sieve were two species of crabs, five species of gastropod, two species of bivalves, hermit crab and nemertea (Table 5.1).

**Table 5.1** Animals found at study site

<b>Animals counted visually in quadrat</b>	
<b>Animals</b>	<b>Scientific name</b>
<b>Crab</b>	<i>Macrophthalmus</i> sp1
	<i>Metaplex crenulatus</i>
	Juvenile crabs ( <i>Metaplex crenulatus</i> )
	<i>Metaplex elegans</i>
<b>Fish</b>	<i>Periophthalmus chrysospilos</i>
<b>Gastropod</b>	<i>Cerithidae cingulata</i>
	<i>Stenothyra</i>
	<i>Nassarius olivaceus</i>

**Animals collected with test sieve (500 µm)**

<b>Animals</b>	<b>Scientific name</b>
<b>Crab</b>	<i>Macrophthalmus</i> sp1
	<i>Metaplex crenulatus</i>
	<i>Metaplex crenulatus</i> (Juvenile)
<b>Gastropod</b>	<i>Cerithidea cingulata</i>
	<i>Stenothyra</i>
	<i>Natica</i> sp.
	<i>Polynices</i> sp
	<i>Littorina scabra</i>
<b>Bivalve</b>	<i>Limnoperna mangle</i>
	<i>Tellina</i> sp.
<b>Hermit crab</b>	<i>Clibanarius infraspinatus</i>
<b>Worm</b>	Nemertea (ribbon worms)



**a.** *Periophthalmus chrysospilos*



**b.** *Macrophthalmus* sp.1



**c.** *Metaplex crenulatus*



**d.** *Metaplex crenulatus* (Juvenile)



**e.** *Metaplex elegans*



**f.** *Cerithidea cingulata*



**g.** *Polynices* sp.



**h.** *Nassarius* sp.





i. *Stenothyra* sp



j. *Littoraria scabra*



k. *Limnoperna mangle*



l. *Tellina* sp.



m. *Nemertea* (ribbon worms)



n. *Clibanarius infraspinus*

**Figure 5.22 (a-n)** Some example of beach fauna found in study site.

### 5.5.2 Effects of biocidal chemicals on epifauna observed on sediment.

The density of crabs, fish and gastropods under the different chemical treatments was significantly different ( $p < 0.05$ ; Appendix 8a-8i). The mean density of crabs in the vicinity of trees treated with distilled water (control) (4.95 no. ind/ m<sup>2</sup>) was significantly higher than in quadrats treated with Ivermectin (2.05 no. ind/ m<sup>2</sup>) and Clorox (1.63 no.

ind/ m<sup>2</sup>;  $p < 0.05$ ). The density of mudskippers in areas where seedlings were treated with Ivermectin (3.06 no./m<sup>2</sup>) was significantly higher compared to that in the control area (2.35 no./m<sup>2</sup>) and in areas where seedlings were treated with Clorox (2.41 no./m<sup>2</sup>). However, the density of mudskippers in the control area was not significantly different compared to the areas where seedlings were treated with Clorox ( $p > 0.05$ ). The density of gastropods in areas where seedlings were treated with Ivermectin (152 no./m<sup>2</sup>) was significantly higher compared to the control area (123.5 no./m<sup>2</sup>) and Clorox (101 no./m<sup>2</sup>). This indicates that Ivermectin also did not harm the population of gastropods. However, the density of gastropods in the control group was significantly higher compared to areas where seedlings were treated with Clorox ( $p < 0.05$ ; Table 5.2).

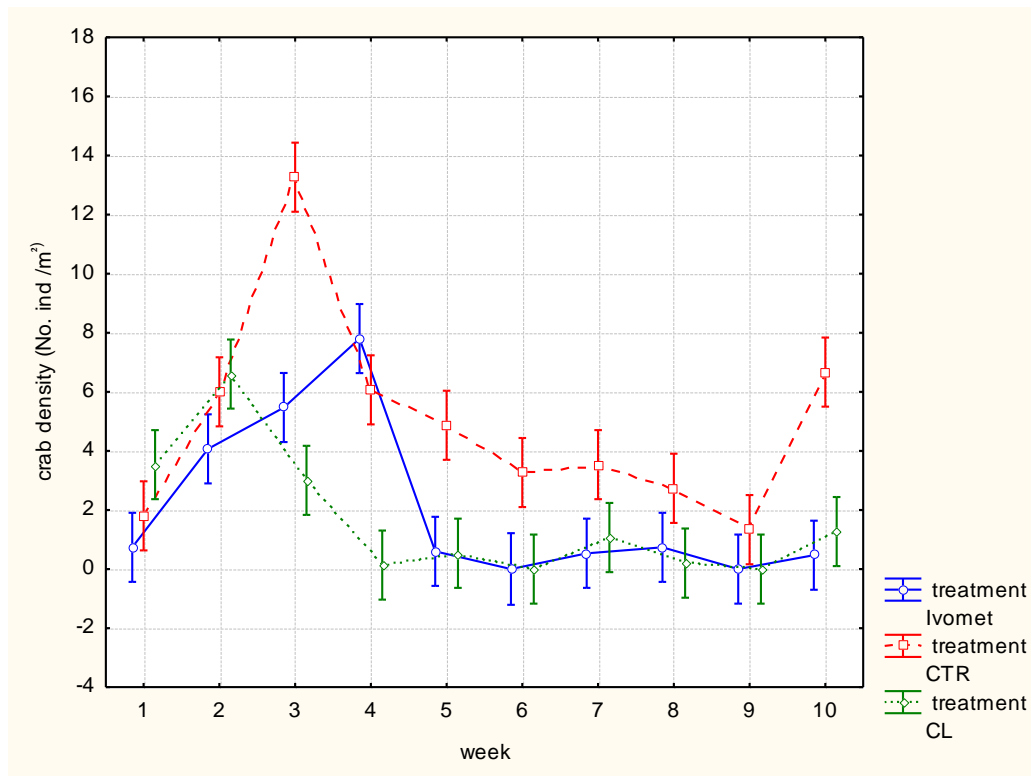
**Table 5.2** The density (mean  $\pm$  SD) of crabs, fish and gastropod in areas where seedlings were treated with Ivomet (Ivermectin), CL (Clorox) and CTR (control, distilled water ) after 10 weeks.

Epifauna	Control			p
	(distilled water)	Ivermectin	Clorox	
Crab	4.95 $\pm$ 5.63	2.05 $\pm$ 2.61	1.63 $\pm$ 2.07	<0.05
Fish	2.35 $\pm$ 1.77	3.06 $\pm$ 2.05	2.41 $\pm$ 1.63	<0.05
Gastropods	123.5 $\pm$ 64	152 $\pm$ 88.7	101 $\pm$ 40.1	<0.05

### 5.5.3 Interaction effects of time and treatment on density of crabs

The interaction effect of time and treatment on density of crabs was significantly different ( $p < 0.05$ ). The density of crabs in areas treated with chemicals was not significantly different in the 1<sup>st</sup> and 2<sup>nd</sup> week ( $p > 0.05$ ; Figure 5.23). The density of crabs in chlorine treated area and the distilled water (control) area decreased significantly

after the 3<sup>rd</sup> week ( $p<0.05$ ). The density of crabs in areas where seedlings were treated with the Ivermectin decreased significantly after the 4<sup>th</sup> week ( $p<0.05$ ). However, the density of crabs in area treated with distilled water was significantly higher compared to areas where plants were treated with Clorox and Ivermectin in the 3<sup>rd</sup>, 5<sup>th</sup> to 8<sup>th</sup> and 10<sup>th</sup> week ( $p<0.05$ ).

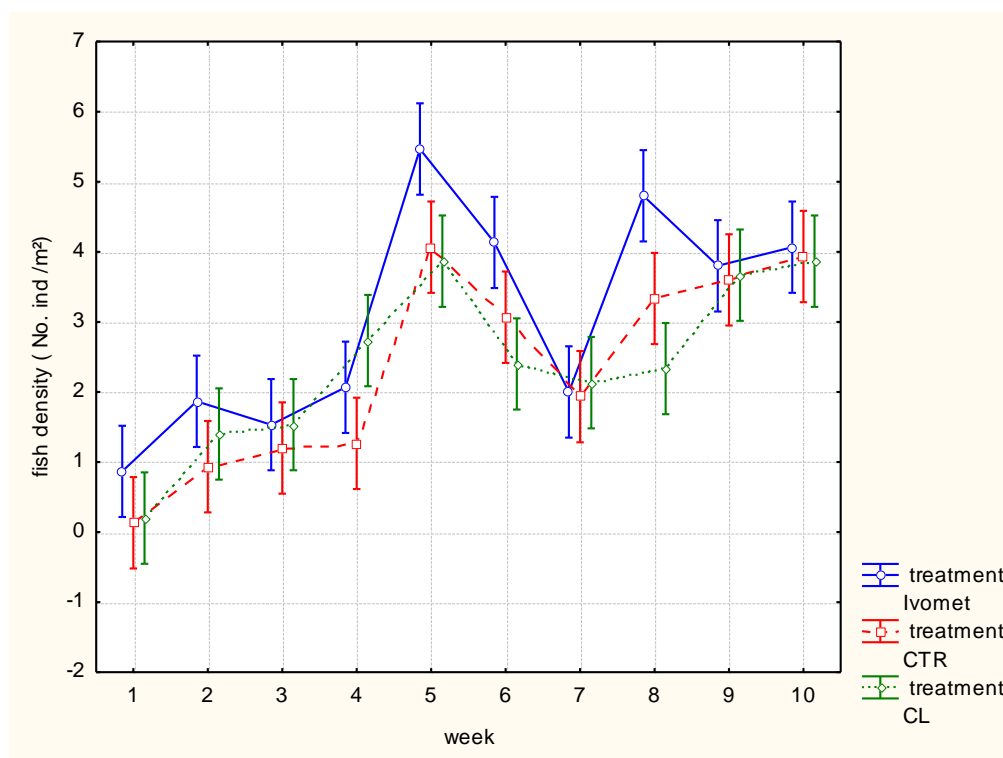


**Figure 5.23** The density (no./m<sup>2</sup>) of crabs in areas where seedlings were treated with Ivomet (Ivermectin), CTR ( distilled water, control) and CL (Clorox) in a period of 10 weeks. Verticals bars denote standard error.

#### 5.5.4 Interaction effects of time and treatment on density of mudskippers.

The interaction effect of time and treatment on density of mudskippers was significantly different ( $p<0.05$ ). For the first three weeks, there were no significant difference ( $p>0.05$ ; Figure 5.24) in mudskippers abundance among areas chemically treated or untreated (control). However in the 5<sup>th</sup> week, mudskipper abundance increased

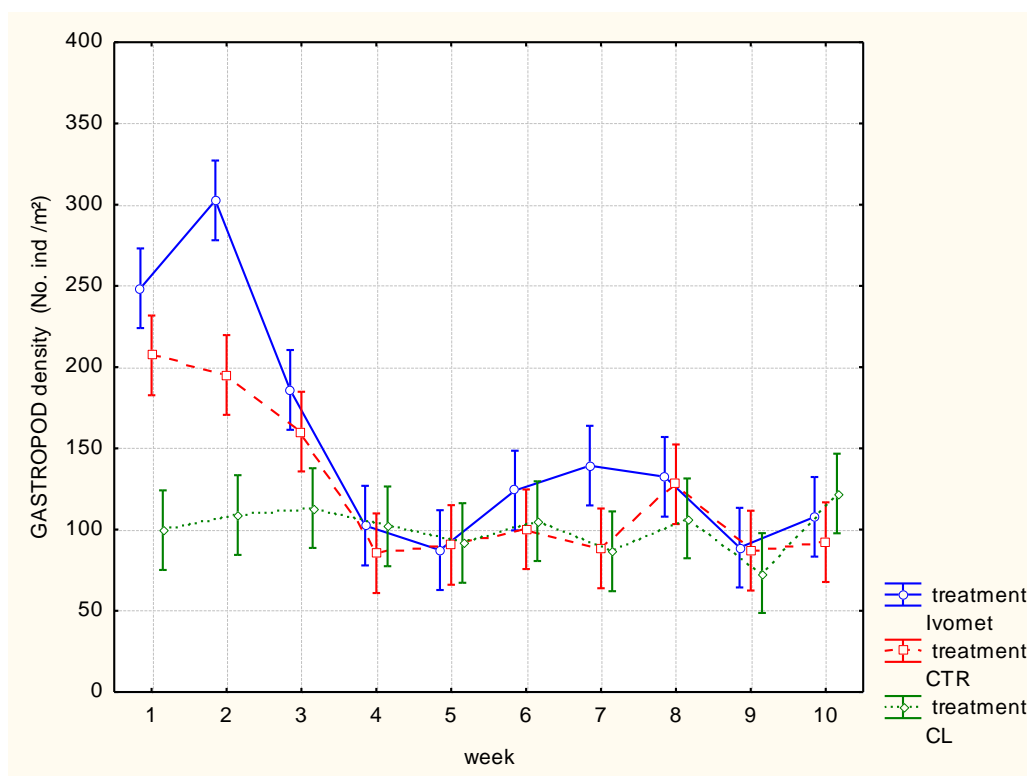
significantly and was higher in Ivermectin treated areas as compared to Clorox treated and control area, except week 7.



**Figure 5.24** The density (no/m<sup>2</sup>) of mudskippers in areas where seedlings were treated with Ivomet (Ivermectin), CTR ( distilled water, control) and CL (Clorox) in a period of 10 weeks. Verticals bars denote standard error.

### 5.5.5 Interaction effects of time and treatment on density of gastropods

The interaction effect of time and treatment on the density of gastropod was significantly different ( $p < 0.05$ ). The density of gastropods in areas where seedlings were treated with Ivermectin and the control group decreased significantly after the 3<sup>rd</sup> week ( $p < 0.05$ ; Figure 5.25). However, for all treatments the density of gastropods areas did not showed significant difference in period between 4<sup>th</sup> week until the 10<sup>th</sup> week ( $p > 0.05$ ).



**Figure 5.25** The density (no/m<sup>2</sup>) of gastropods in areas where seedlings were treated with Ivomet (Ivermectin), CTR (distilled water, control) and CL (Clorox) in a period of 10 weeks. Verticals bars denote standard error.

### 5.5.6 Infauna collected with test sieve.

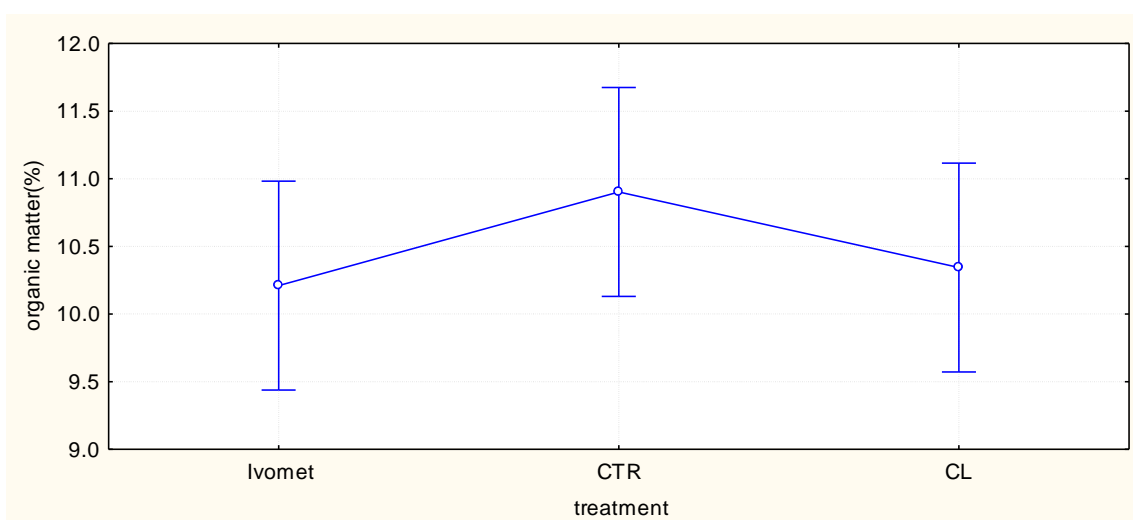
The density *Tellina* sp, Nemertea and crabs among area give the three chemical treatments was not significantly different ( $p < 0.05$ ; Table 5.3).

**Table 5.3** The density (mean  $\pm$  SD) of *Tellina* sp, Nemertea and Crabs in areas where seedlings were treated with Ivomet (Ivermectin), CL (Clorox) and CTR (control, distilled water) after 10 weeks.

Control				
infauna	(distilled water)	Ivermectin	Clorox	p
<i>Tellina</i> sp.	0.05 $\pm$ 0.12	0.02 $\pm$ 0.07	0.05 $\pm$ 0.13	>0.05
Nemertea	0.01 $\pm$ 0.04	0.01 $\pm$ 0.06	0.01 $\pm$ 0.04	>0.05
Crabs	0.02 $\pm$ 0.08	0.02 $\pm$ 0.07	0.04 $\pm$ 0.12	>0.05

### 5.5.7 Effect of biocidal chemicals on organic matter content of soil

The effect of chemicals, time and interaction effect on the organic matter content of the soil (Ivomet=10.21%, Clorox=10.34% and Control=10.21%) was not significantly different ( $p>0.05$ ; Figure 5.26). The present study did not show any serious effects of the chemicals on the organic matter of the soil.



**Figure 5.26** The organic matter content of soil in the areas where seedlings were treated with Ivomet (Ivermectin), CTR (control) and CL (Clorox).

## CHAPTER 6: DISCUSSION AND CONCLUSION

### 6.1 Colonization of barnacles on *Avicennia marina* seedlings (Experiment 1).

The mean abundance of the barnacles on *Avicennia marina* seedlings with heights that ranged from 40-50cm during a period of 8 week was  $397 \pm 269$  no./plant (Figure 3.3). The abundance of barnacles on *Avicennia marina* seedlings was different with time. Nonetheless, within the first month of settlement, the total numbers of barnacles on the seedlings fluctuated greatly especially on the leaves (Table 3.3). Heat, desiccation, predation and bulldozing effect from mobile mollusks may affect the pre-settlement of barnacles on the seedlings (Apolinário, 1999). During the 5<sup>th</sup> week to 8<sup>th</sup> week, the population of barnacles became relatively consistent. A possible explanation could be that the mature barnacles were more firmly attached on mangrove seedlings.

The abundance of barnacles on the different sections (or height above ground) of the seedling was significantly different ( $p < 0.0001$ ). The segment S20 (stem 10-20cm height above ground) had the highest mean number of barnacles (125 no./plant;  $p < 0.05$ ; Figure 3.4). S20 was located close to the mud surface and thus exposed to a relatively higher humidity as compared to the upper sections of the plants. The cyprids of barnacles may tend to avoid lethal heights and dryness during the phase of permanent attachment (Hills *et al.*, 1998). The settlement in the above segment could be due to percentage cover by tides. Segments above 20cm of the stem may not be suitable for barnacles growth due to inadequate tidal cover for their feeding and respiration (WetHEY & David, 1984).

Although the S10 segment was the region with the longest submersion time by sea water, the variable level of the soft mud might have intermittently covered the biofouling organisms and hence reduced their abundance unlike S20 which was higher up. A study in Australia had shown that both barnacles and copepods died after less than one hour of exposure to high concentration of suspended mud (Fabricius & Wolanski, 2000). Leaves and sections of 30cm-40cm and 40cm to 50cm above ground had lesser number of biofouling organisms due to their lower submerged period in sea water.

The percentage cover of barnacles on different sections of the stem and leaves was significantly different ( $p < 0.05$ ). In the first month, barnacles had covered more than 30% of the bottom half of the stem (Figure 3.5). As the abundance of barnacles increased, it contributed to the increase in percentage cover on the mangrove seedlings. Thus S20 had a significantly higher percentage cover of barnacles (47.6%) compared to other sections ( $p < 0.05$ ). The percentage cover of barnacles on the lower leaf (16.9%) was significantly higher compared to the upper leaf (8.89%; Figure 3.6). This can be explained by the different texture of the leaves. The lower leaves have prominent veins and rougher surface compare to upper leaves. The upper leaf surface was also exposed to stronger sunlight and desiccation. A study in India showed that exposure to a temperature of 38°C would cause 100% mortality of 9 mm size group mussels in 120 min. All sizes of these mussel groups showed a progressive reduction in physiological activities such as filtration rate, foot activity and byssus thread production when temperature was increased to 30°C. (Rajagopal *et al.*, 1995).

Many factors have been shown to affect the settlement of barnacles. Hill and Thomason (1996) had included tidal and hydrodynamics, with larvae patchiness in the



water column (Hawkins & Hartnoll, 1982) or the tidal height being the most important factor of passive settlement process (Wethey & David, 1984). The physical, chemical and biological aspects of substrata (Maki *et al.*, 1988) and conspecific effects (Larman & Gabbott, 1975) will also affect the settlement of barnacles. Wethey (1984) found that surface contour is far the dominant influence on settlement density of the barnacle *Semibalanus balanoides* (Yule & Walker, 1987). Texture has a strong effect on settlement pattern as well as settlement density of *S. balanoides* cyprids (Hill & Thomason, 1998a). The larvae of barnacles respond to the surface with a similar scale to their body size (0.5-2mm; Hill & Thomason, 1998a). A similar effect was described by Bourget *et al* (1994) for *Mytilus edulis* and *Hiatella arctica* which settle in relation to surface components at about the 1 mm scale, similar in size to their body size at settlement. The need to settle in refuges is possibly determined by maximizing adhesion in a low shear environment (Walters & Wethey, 1996), or some others physical requirement, such as prevention of desiccation (Raimondi, 1990). However, other factor such as larva availability (Minchinton & Scheibling, 1991) and mortality (Raimondi, 1990) could also change over time and confound the comparisons between the rapidly and slowly settled of the barnacles (Hill & Thomason, 1998a).

*Amphibalanus amphitrite* were hermaphrodites which possess both male and female reproductive organs (Charnov, 1987). It allow self-fertilization to produce offspring when there are no other barnacles nearby (Barnes & Crisp, 1956) if two barnacles happen to be close to one another, one will protrude a long penis into the other barnacle. The penises of *balanus amphitrite* can extend up to eight times its own body length (Darwin, 1854c). Individuals release as many as 10,000 eggs per brood, with as many as 24 broods per year (El-Komi & Kajihra, 1991). Metamorphic life cycle starts out as a free-swimming larva, called a nauplius. In *Balanus amphitrite*, the six

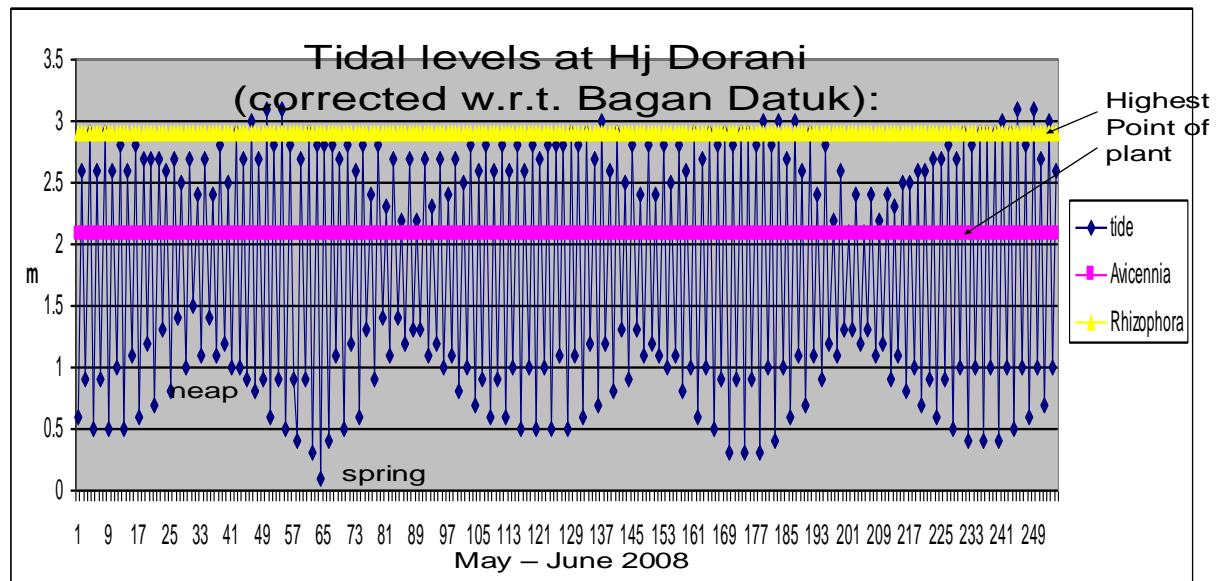
ecdyses from the newly released stage I nauplius to the cyprid are completed within 5 days at 28°C, under laboratory conditions (Hellio *et al.*, 2004). Prior to settlement, cyprids navigate from the water column to potential settlement sites, first exploring them using a temporary adhesive system and then attaching permanently with a discrete adhesive termed the ‘cyprid cement’ (Phang *et al.*, 2006) or permanent cyprid adhesive. The cement-like secretion is similar to the proteins of humans blood clot (platelets) (Dickinson *et al.*, 2009). Numerous surfaces may be explored and rejected before the cyprid either locates a suitable surface or becomes sufficiently ‘desperate’ to select a surface with less favourable characteristics (Elkin & Marshall, 2007). Cyprids are highly discriminatory during exploration and judge a surface’s suitability based on criteria including texture (Schumacher *et al.*, 2007), local hydrodynamics (Eckman *et al.*, 1990; Koehl, 2007), surface chemistry (Aldred, 2007), surface colour (Yule & Walker, 1984) and the presence of adult or cyprid conspecifics (Yule & Crisp, 1983).

Knight-Jones (1953) was the first to observe that a water-soluble protein, later termed ‘arthropodin’, originating from adult barnacles, was important for the gregarious settlement of *Semibalanus balanoides* cyprids. The integument of adult barnacles (and extract thereof) is known to induce conspecific settlement in some species (Knight-Jones, 1953; Yule & Crisp, 1983). This inducer of gregariousness is now termed the settlement-inducing protein complex (SIPC) (Matsumura *et al.*, 1998). Through assessment of surfaces using innate criteria, cyprids are able to identify those that manifest advantageous physical and environmental conditions, and that have an established population of conspecifics. In selecting and attaching permanently to these surfaces they give themselves the best chance for survival and reproduction (Aldred & Clare, 2008).

## 6.2 Colonization of barnacles on *Rhizophora apiculata* seedlings (Experiment 2)

The abundance of barnacles on the *Rhizophora apiculata* seedlings was very low with an average of not more than 5 barnacles per plant (Figure 3.7) after two months from planting. These results were different compared to a study in India which showed that *Amphibalanus amphitrite* preferred to attach on *Rhizophora apiculata* as compared to *Avicennia marina* (Rani *et al.*, 2010a). The author stated that barnacles tend to attach on the rougher surface of the *Rhizophora apiculata* compared to the smoother surface of *Avicennia marina*. There are however other reasons why fewer barnacles attach themselves onto *Rhizophora apiculata* as compared to *Avicennia marina* seedlings in the present study. Firstly, the *Rhizophora apiculata* seedlings were planted on the upper shore (2.5m above C.D; Figure 2.2; Figure 3.1), whereas the *Avicennia marina* were planted on the lower shore (1.6m above C.D). During certain days at neap tide, the sea water would not reach up to the high shore (Figure 6.1). In addition to that, the environment at the upper shore area was dryer and hotter as compared to the lower shore area. Hence, it would be difficult for cyprids to survive in such an extreme environment (Hills *et al.*, 1998). Secondly, the *Rhizophora apiculata* seedlings were planted behind the geo-tubes. The geo-tubes (Figure 2.3) have caused the deposition of mud which increased the elevation of the shore behind it. Thirdly, the *Rhizophora apiculata* seedlings were planted on coir-logs (Figure 1.3) which effectively raised the height of the seedlings by 15-20cm above ground. Thus the plant top would be less submerged and this reduced the settlement intensity of barnacles on the seedlings. S10 of *R. apiculata* had a longer submersion period in sea water, and thus the abundance of barnacles was significantly highest (1.12 no./plant) compared to the other sections (Figure 3.8). *Avicennia marina* seedlings planted below mean high water level were more frequently submerged during both neap and spring tides (Figure 6.1). High

submersion frequency has been reported to increase the intensity of biofouling on mangrove seedlings (Olivier *et al.*, 2000).



**Figure 6.1** Tidal levels at KSHD from May until Jun 2008. The plotted tidal levels clearly show that while *Rhizophora* seedlings on higher ground were only submerged during the high spring tides (yellow line), *Avicennia* seedlings were submerged during both spring and neap tides (pink line).

### 6.3 Effects of biofoulers on growth and survival of a replanted mangrove seedlings.

In various studies on mangrove plantations, fouling by barnacles has been reported to affect the survival of planted seedlings (Ellison & Farnsworth, 1990; Macintosh & Ashton, 2002). Tides, current and high density of barnacles are the main causes of seedling death in Malaysia (Anonymous, 2010). *Amphibalanus amphitrite* was the main fouling organism found on the mangroves (Rani *et al.*, 2010a). In this study, barnacles (especially *Amphibalanus amphitrite*) were also the dominant biofouling organisms (more than 90%) on replanted mangrove seedlings (Figure 3.2a). *Amphibalanus amphitrite* do not absorb nutrients from mangrove seedling. It feed on small and micro-sized particles like bacteria, plankton and waste nutrients. It feed when submerged or

exposed to water (during high tides). The operculum opens and cirri exposed capture food. The cirri retract into shell and operculum is closed when not exposed to water (exposed to air during low tide). It capture prey by grabbing or filtering small particles uses foot-like appendages (cirri) which are attached to their limbs cirri placed perpendicular to the direction of water currents. Cirri swiped in and out of oral cavity at high velocity, 'grabbing' food particles in a repetitive manner (Crisp & Southward 1961). When water current is slow, cirri are placed above the oral cavity (at different directions) similar to a mesh trap; retraction of cirri into oral cavity is slow (Crisp & Southward, 1961). Barnacles growth on the leaf surface presumably reduces photosynthesis (Maxwell, 1993; Figure 4.1j). Santhakumaran (1994) stated that a high density of barnacles will create a physical drag and weight on plant, causing leaves to fall and stem breakage. The roots are important because they are the support system and hence any blockage of root growth could eventually cause the seedling to collapse. For *Avicennia* sp. without its pneumatophores roots, the plant may not obtain enough oxygen from the mud (Scholander *et al.*, 1955). According to Satumanatpan (1999), seedlings with barnacles attached on the stem grew taller than those that had barnacles removed from the stem. However the seedlings grew almost twice as many new leaves if barnacles were removed from the upper leaf surfaces. When the growth profile of mangrove was studied for two years, she found no significant effect either by the presence or absence of barnacles. In Hong Kong, Li and Chan (2008) had reported that fouled plants did not have hanging propagules.

Gastropods (*Littoraria* spp.) found on replanted mangrove seedlings were *Littoraria scabra* and *Littoraria melanostoma* (Figure 4.1k; 4.1l). A study in Australia states that the pressure of gastropods only results in minor impact to mangrove (Clarke & Allaway, 1993). Lii (1987) reported that some herbivorous gastropods may reduce

the survival of mangrove seedlings. The snail moves up the tree to avoid flood water and predation, and downward during ebb tide to feed. It is a generalist herbivore, feeding preferentially on microalgae and bacteria, but easily shifts its diet by feeding on a variety of foods such as leafy macrophytes, filamentous algae, mangrove tissues and even zooplankton (Alfaro, 2008). However, snails that glide on the surface of mangrove seedlings may help to push away the attached cypris of barnacles and indirectly reduce the number of barnacles on seedlings (Apolinário, 1999).

Mussels grew among barnacles (Figure 4.1m). *Limnoperna mangle* (mussel) may increase the burden on young mangrove seedlings and prevent new leaves and roots from sprouting (Ong *et al.*, 2010). A mutualistic interaction between barnacles and *Morula* (a gastropod preying on mussels) has been reported by Lively and Raimondi (1987). Barnacles will attract the settlement or enhance the survival of the mussels, while *Morula* reduces the competitive effect of mussels on barnacles.

One of the effects of biofouling on the environment is the introduction of non-indigenous species (NIS). NIS may affect the substrate itself, or alter habitat architecture, indirectly influencing water movements, sediment accumulation and light conditions (Wallentinus & Nyberg, 2007).

The effect of time (week) on the number of leaves of *Avicennia marina* was not significantly different ( $p > 0.05$ ; Figure 4.2; experiment 1). This can be explained by the slow growth rate of transplanted seedlings. A study in Australia showed that seedlings of the *Avicennia marina* only increased by 2 cm total length and grew a few additional

leaves after one year of observation (Satumanatpan & Keough, 1999). The newly transplanted seedling may need more time to adapt to the new environment.

The effect of time on the number of leaves of *Rhizophora apiculata* was significantly different ( $p < 0.001$ ; Figure 4.3; experiment 2). The number of leaves in the 9<sup>th</sup> week was significantly higher compared to others except in the 6<sup>th</sup> week ( $p < 0.05$ ). The number of leaves had increased showing that the seedlings were growing.

The average diameter of the *Amphibalanus amphitrite* on the seedling stem was  $4.48\text{mm} \pm 0.75$  in the 7<sup>th</sup> week. The size was much smaller compared to the barnacles found on the rocky shore in which the maximum shell diameter was 16.4mm to 16.9mm (Calcagno *et al.*, 1997). The high competition for space may limit the barnacle size (Lohse & Raimondi 2007). Crowded individuals were much smaller than uncrowded individuals (Lively & Curtis, 1986). When settlement density is sufficiently high, it results in mounds known as hummocks (Connell, 1961). However, the size of barnacles on the leaves was found to be smaller than on the stem. This is because the species attached on the leaves were star barnacles (*Euraphia withesis*), whereas those attached on the stems were acorn barnacles (*Amphibalanus amphitrite*). Interspecific competition for space will affect the abundance and distribution of barnacles (Connell, 1961). The lower inundation of leaves may also affect the nutrient supply to barnacles which are thus smaller (Lohse & Raimondi, 2007).

Results in Experiment 3 indicate that the development of barnacles on the seedlings were not dependent on the species of mangrove seedlings (*Avicennia marina*,

*Rhizophora mucronata* and *Rhizophora apiculata*) but appeared to be due to physical factors, in this case, the height of the plant above the ground; the lower the height, the longer the submersion time, and thus the higher the percentage cover of barnacles. The growth and survival of seedlings with low percentage cover (<30%) of barnacles and other biofouling organisms were not affected over the 20 weeks of study.

In the histological study of plant tissues, the leaf sections that were fouled had significantly lower mean percentage thickness of palisade layer compared to that of non-fouled leaves sections. However, the effect of barnacles on the number of abaxial hairs was not significantly different. The results should however be taken with caution since this is a localized response given that the parameters were measured on the same leaf at fouled and non-fouled surfaces. The barnacle effect was not tested at the leaf or plant level where a more general response is expected. Nonetheless, leaf shading could induce a reduction in the palisade layer of the entire leaf. Ashton & Berlyn (1992) found that leaves of the seedlings of four species of the genus *Shorea* exposed to higher light intensity had thicker palisade as compared to shaded leaves. According to Li & Chan (2008), the reduced thickness of the palisade layer and the increase in number of abaxial hairs in the leaves of fouled mangrove plants could be an indirect effect of increased stomata density. However, increased stomata density in a fouled tree may be a response to stress (Li, 2005). Also, the reduction in palisade layer thickness could reduce the number of chloroplasts (Lee *et al.*, 1990). Thus, in the long term, barnacle infestation likely contributes to eco-physiological stress of the plant such as reduction in photosynthesis and gaseous exchange (Maxwell, 1993).



The cement bases of *Amphibalanus amphitrite* were found on the surface of stems (Figure 4.1e; Figure 4.10.). Adhesive cement produced by barnacles on stems may be harmful to tree or shrub growth (Santhakumaran & Sawant, 1994). The calcareous base of *Amphibalanus amphitrite* is impermeable to gas and its impact will continue to be left on the plant's surface even after the barnacles had died, leaving the empty shell (Li & Chan, 2008). In contrast, *Euraphia withersi* commonly found on the leaves may impact less detrimentally to the mangrove seedlings. These barnacles have membranous bases (Figure 4.1c; Figure 4.1d). When *Euraphia withersi* dies, the base will dislodge. A study in Australia, showed that the impact of *Elminius* sp. (barnacles) on the growth of mangrove seedlings was not significant because *Elminius* has a membranous base (Satumanatpan & Micheal, 1999).

#### **6.4 Study on using physical barrier to reduce biofouling organisms on replanted *Avicennia marina* seedlings (Experiment 4)**

The mean abundance of barnacles found on seedlings using the conventional method (1223 indiv./plant) was significantly higher compared to the physical barrier method

(18 indiv./plant) in a period of 10 weeks (  $p < 0.05$ ; Figure 5.1). These results support the hypothesis that the physical barrier method is able to reduce the growth of fouling organisms on mangrove seedlings and provides a better performance in terms of mangrove seedling survival and growth. The physical barrier method using PVC pipings is able to keep out the larvae of foulers. A study in the Indian River Lagoon showed that the mangrove seedlings planted in a full length PVC encasement had highest survival rate and growth as compared to the half-length PVC pipe and conventional method (Kent, 1999).

Strong currents, tidal waves and soil erosion were reported to affect the survival rate of mangrove seedlings (Satumanatpan & Keough, 1999). The PVC pipe has inert properties, easy access, low cost, strength and durability against strong waves and able to reduce the impact of natural factors on the seedlings' survival (Riley & Kent, 1999). Casement methods have been shown to be also effective and protective for nutrient-rich seedlings from being consumed by animals (Moore, 2004; Satumanatpan & Keough 1999). However, it is necessary to remove the PVC piping after the roots have established.

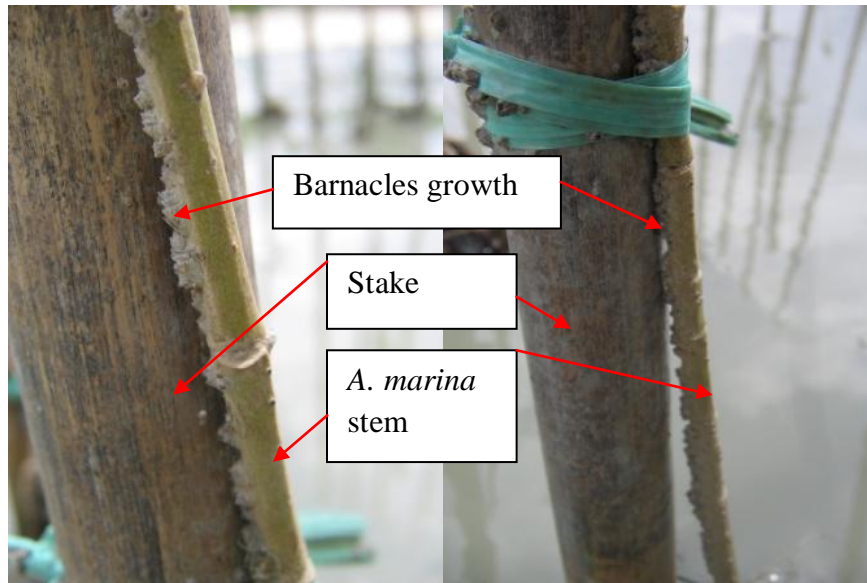
### **6.5 Study on effectiveness of the elevated and short stake method of planting to reduce biofouling on *Avicennia marina* seedlings (Experiment 5)**

Based on the findings from experiment 1, 2 and 4, an experiment was conducted to test the relationship between biofouling and the planting method. The mean abundance of barnacles in the conventional method (21.3 no./plant) was significantly higher compared to the elevated method (1.5 no./plant) and the short stake method (5.5 no./plant; Figure 5.7). These results support the hypothesis that mangrove seedlings that are less frequently submerged in water will have less biofouling. Cyprids are likely to avoid lethal height above ground and dry areas for permanent attachment (Hills *et al.*, 1998). This result also supports the hypothesis that the fastened seedling against a stake will encourage more settlement of barnacle larvae on the seedlings. This is because the fastened seedlings and the stake form a gap in between them. This gap or space is a zone of reduced current speed that would provide a calm zone for settlement and adherence of cyprids (Figure 6.3). High settlement of biofouling larvae occurs when the

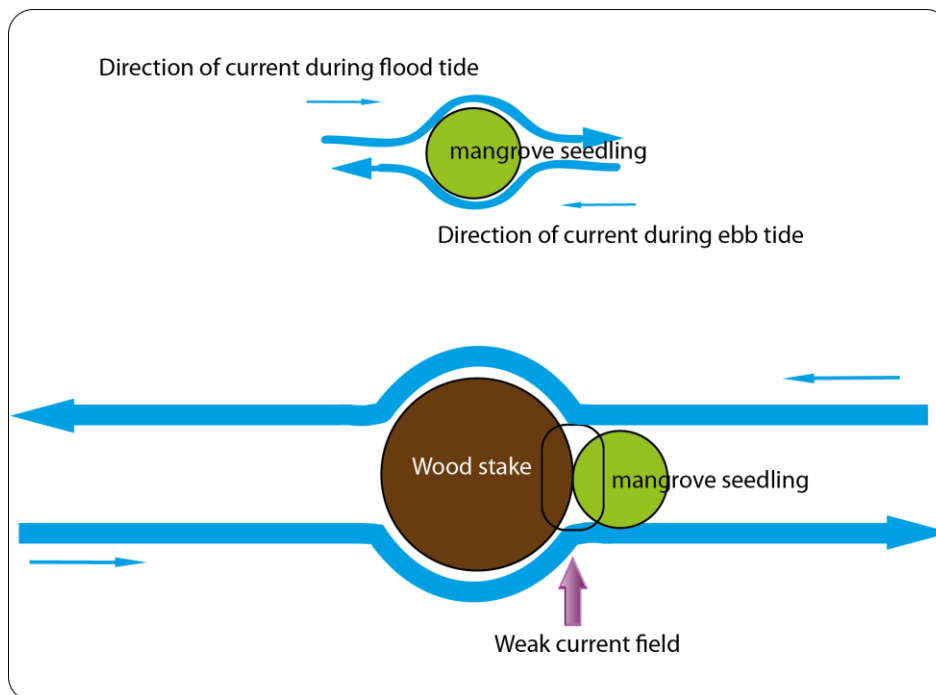
water current speed is less than 25cm/s (Madin & Chong, 2010). The gap between the seedling and the stake further provides the shading effect and thus reduce the lethal effects of heat and desiccation on cyprids. Settlement areas with a rougher surface may provide protection against the swift water current that could sweep larvae away. Surface irregularities may change the surrounding abiotic conditions, for example, increase moisture, reduce direct sunlight and air exposure, besides lowering the water movement (Skinner & Coutinho, 2005).

A study in the United Kingdom had shown that the settlement of barnacles in cracks was ten times greater than would be expected if there was no preference for cracks (Wetthey, 1984). Because adults are sessile, finding a good spot to settle (the location it will live in for the rest of its life) is very critical (Aldred & Clare, 2008).

The number of leaves on the seedlings planted using the elevated method is also significantly higher compared to seedlings planted using the short stake method and conventional method ( $p < 0.05$ ; Figure 5.10). The design for the elevated method can reduce the impact of soil erosion as compared to the conventional method and short stake method (Figure 5.15a). Soil erosion can expose the roots of the seedlings planted using the conventional and short stake method and eventually the seedlings dry up and die (Figure 5.13a; Figure 5.14a).



**Figure 6.2** The gap between stake and seedling allows the settlement of barnacles. Note the settlement of barnacles in between stake and stem.



**Figure 6.3** An explanation for barnacle settlement between stake and stem of mangrove plant (cross section) due to the zone of weak current field (rectangle).

## 6.6 Effects of chemicals on biofouling organisms and *Avicennia marina* seedlings (Experiment 6 and 7).

The mean abundance of barnacles in the control group (95.3 no./plant; Figure 5.20) was significantly higher compared to the others (tea seed cake, Clorox, Ivermectin, Neguvon) ( $p < 0.001$ ). However, all these chemicals were unable to totally prevent biofouling. This is due to the removal or dilution of chemicals during high tide (inundation) or rain. The general uses of chlorine include water treatment for protection of public health and industrial uses for anti-fouling (Brungs, 1973). Chlorine is effective in reducing biofouling at the level of concentration of  $\geq 0.5$  mg/l in sea water (Saeed, 2002). A concentration of 2.5 mg/l of chlorine residual could cause a population mortality of as high as 80 percent of the barnacle nauplii (*Balanus* sp.) and 90 percent in the copepod (*Acartia tonsa*) during a 5-min exposure (McLean, 1973). Active chlorine concentrations as high as 1000 ppm are necessary for a substantial reduction of bacterial numbers in a biofilm, whereas 10 ppm is sufficient for planktonic cells (Meyer, 2003).

In experiment 7, chlorine10 (100% clorox) was the most effective to reduce biofouling compared to chlorine1 (10% clorox mix with 90% of distilled water) and chlorine5 (50% of clorox mix with 50% of distilled water; Figure 5.20). However the number of leaves growing on the mangrove seedlings in chlorine10 was significantly reduced compared to the control group (Figure 5.21.). A study in US reported that phytoplankton exposed to chlorination had depressed rates of photosynthesis and respiration (Brook & Baker, 1972). Thus the high concentration of chlorine may affect seedling health and growth.

Ivermectin is an anti-parasitic drug effective against a wide variety of parasites (Burridge & Haya, 1993). Ivermectin has been shown to be very effective against sea

lice (crustacean ectoparasites, *Caligus elongatus* Nordmann and *Lepeophtheirus salmonis* Kroyer), infestations of Atlantic salmon (Smith *et al.*, 1993). The LC50 value of Ivermectin exposed mysid shrimp *Neomysis integer*, was shown to be 70 ng/l in a study conducted in UK. The mussels, *Mytilus edulis* exposed to Ivermectin in water at a concentration of 6900 ng  $^{-1}$  for 6 days showed a bioconcentrated Ivermectin level increase with an estimated depuration half-time of 22 days (David *et al.*, 1997). The present study showed that the abundance of biofouling at all given concentrations of Ivermectin (Figure 5.20) was significantly lower compared to the control group ( $p < 0.05$ ). This indicates that Ivermectin was effective in controlling biofouling. The number of leaves of seedlings treated with Ivermectin was not significantly affected, compared to the control group (Figure 5.21) indicating that Ivermectin did not disturb seedling growth.

Neguvon is sold as a soluble powder, which can be added directly to water and is highly effective against crustacean ectoparasites and some monogeneans (Syndel, 2009). An active ingredient of Neguvon is trichlorfon (97%). A bath treatment of 300 ppm of Neguvon for 15–60 min is therapeutically effective against the parasitic copepod *Lepeophtheirus salmonis* (Brandal & Egidius, 1979). In another study, survival tests that were carried out on the lobster (*Homarus gammarus*) held in tanks of flowing seawater gave total mortality within 24 hours when animals were exposed to Neguvon at a concentration of 0.5 ppm (Egidius & Møster, 1987). The present study showed that the abundance of biofouling organisms at all concentrations of Neguvon was significantly lower than the control group ( $p < 0.05$ ; Figure 5.20). The numbers of seedling leaves treated with Neguvon1 (0.97mg trichlorfon/ml) and Neguvon 10 (9.7mg trichlorfon/ml) was significantly lower compared to that of the control group (Figure

5.21.), indicating that Neguvon may negatively affect seedling health. As such, this chemical is not recommended for the control of biofouling on mangrove seedling.

## **6.7 Effects of antifouling chemicals on beach fauna at study site (Experiment 8)**

Swain (1998) shows that even extremely low concentrations of the tributyltin moiety (TBT) will cause defective shell growth in the oyster *Crassostrea gigas* (20 ng/l) and imposex in the dog-whelk *Nucella* sp. (1 ng/l). Thus it is necessary to find alternative biocides which do not seriously impact the environment.

The abundance of crabs in areas where seedlings were treated with Ivermectin and chlorine were significantly lower compared to the control group area ( $p < 0.05$ ; Table 5.2). Both crabs and barnacles are crustaceans which are vulnerable to the tested chemicals. Ivermectin kills invertebrates by adversely affecting neurotransmission through the disruption of chloride ion movement as a result of irreversible competitive binding to glutamate-gated chloride channels (Arena *et al.*, 1995). The effects of Ivermectin has been shown to be limited to arthropods and nematode parasites (Campbell & Benz, 1984). Burrige (1993) stated that a nominal 96 h LC<sub>50</sub> mortality of 8.5  $\mu\text{g}$  Ivermectin per gram of food is dangerous to shrimps. Any higher concentration may present a hazard to non-target organisms during or after oral treatment to fish against parasites (Burrige & Haya, 1993). However, Ivermectin undergoes rapid degradation under light in soil. It will not accumulate and will not undergo translocation in the environment (Halley *et al.*, 1993). Accumulation of Ivermectin in mussels was lower than the detectable concentration (Davies *et al.*, 1997). Results of repeated studies of dosing rats and bluegill sun-fish with Ivermectin also

confirmed that emamectin benzoate (Ivermectin) is not a bioaccumulative compound (Mushtaq *et al.*, 1996).

The abundance of gastropods in Ivermectin<sup>5</sup> was significantly higher compared to seedlings treated with chlorine and control group. This indicates that Ivermectin did not harm the population of gastropods. Abundance of gastropods in control and chlorine treatment showed no significant difference ( $p>0.05$ ). ANOVA showed that the abundance of gastropods near to the control and chlorine group were not significantly different ( $p>0.05$ ). However, a study showed that snails of three freshwater species did not recover after 24 hours exposure to  $2\mu\text{g/ml}$  of Ivermectin at  $28^{\circ}\text{C}$  (Okafor, 1990). Furthermore, sublethal concentrations ( $0.001\text{-}0.01\text{ }\mu\text{g/ml}$ ) were shown to substantially reduce the number of eggs produced by the snails.

Lobster larvae and juvenile killifish showed significant respiratory stress with exposure to chlorine at sublethal levels (Capuzzo *et al.*, 1977). Trees exposed to chlorine showed a reduced mean leaf area, with a higher percentage of leaf area damaged, a reduction in fruit yield, chlorophyll pigments, protein and carbohydrate content, and a higher accumulation of chloride in the foliar tissues (Vijayan & Bedi, 1989).

The present study did not show any effects of the tested chemicals on the organic matter of the soil (Figure 5.26). Ivermectin and chlorine had 10.2% and 10.3% organic matter opposed to control (10.2%;  $p>0.05$ ). This indicates that the chemicals may not affect the in situ soil microorganisms and activity.



Ivermectin<sup>5</sup> and chlorine<sup>5</sup> affected the population of crabs in this study. However, ANOVA showed that these chemicals have no significant effect on animals such as *Tellina* sp, Nemertea and Crabs ( $p < 0.05$ ; Table 5.7). The results of these chemical treatments however show that they are not very effective control methods. The biocidal chemicals applied on the barnacles are unable to exterminate the adult barnacles but do however seem effective against the cypris. The treatment is also very laborious as each seedling has to be regularly applied with the chemical for it to take effect. Also, the long run effects of the chemicals to the environment are unknown and as such a suitable level of chemicals to be applied cannot be concluded. Therefore it is advisable to use chemical treatment as a means of control in the most extreme cases of infestation.

## 6.8 Conclusion

The dominant biofoulers on replanted mangrove seedlings at Kampung Sungai Haji Dorani and Kampung Sungai Limau were barnacles (*Amphibalanus amphitrite*). Frequency of sea water submersion period and planting method were the main factors affecting colonization of biofouling on seedlings.

The high density and coverage of barnacles appeared to be obstructing development of new leaves, shoots and roots, as well as imposing weight loads as high as six times the weight of the seedlings. However, growth and survival of seedlings with low percentage cover (<30%) of barnacles and other biofouling organisms were not affected over the 20 weeks of study. Results from histological study showed that there

was no significant difference ( $p>0.05$ ) in both % thickness in palisade layer and number of abaxial hairs between fouled and non-fouled leaves.

Physical barrier and elevated method were effective in reducing barnacle settlements on mangrove seedlings which had higher survival and number of leaves compared to the conventional method. The short stake method was effective in reducing barnacles but at the same time caused lower plant survivability with lesser number of leaves, as shown also by the conventional method. In the chemical control study, weekly topical applications of Clorox, Ivermectin, Neguvon and tea seed extract can significantly reduce the abundance of barnacle infestations. Although, applications of Ivermectin and Clorox had significantly caused a reduction in crab abundance, nonetheless, abundance of both gastropods and fishes was unaffected.

## **6.9 Further studies**

In a mature mangrove ecosystem, it is found that barnacles normally do grow on the trunk of mangrove trees but they are not as abundant as on replanted seedlings in a rehabilitated site such as KSHD (personal observation). It is deduced that these trees in mature ecosystems may already have their own form of defense against barnacles through natural chemical secretions. However, this needs to be verified by studying mangrove seedlings of species which have natural defense mechanisms, for example by planting and comparing local (with natural defense) and imported species of mangrove plants. As the location of the experiment was conducted in a created or man-made environment, the area would not have a complete ecosystem containing both prey and predator. For this reason, the planting area initially attracts barnacles which are ubiquitous and abundant in the sea water (Ong *et al.*, 2010). Hence, without (lack)

predators, barnacles grow unrestricted. For example, gastropods (snails and slugs) and other crustaceans (like crabs) dog whelks (*Nucella lapillus*) are carnivorous sea snails use special drilling mouth parts (radula) to bore through the shells of barnacles (Largen, 1967). It digestive enzymes are injected through the hole and the resulting liquid food sucked. For a clearer picture on the control of barnacles, the study should ideally be also compared to one that was conducted in the natural ecosystem with both prey and predator present. To show the effect of predation in the natural ecosystem, a native predator species of barnacles (eg. *Thais tissoti*) may be introduced to the replanting site.

## **6.10 Recommendation**

As a recommendation, should the government decide to plant mangrove seedlings below the mean height water level, the barrier or encasement method of control should be used as this would help to protect the seedlings from barnacle infestation. Should the seedlings be planted at a height equal to the mean water height, the elevated method would be sufficient to protect the seedlings from barnacles. Finally, using the conventional method would suffice when planting seedlings at a height above the mean height water level. The use of both enhancement and conventional method depending on the height of the shore (above CD) would also be more economical compared to other planting methods like coir-logs. It is too early to tell the best biocidal chemical to control biofouling organisms on replanted mangrove seedlings. However, results for this study showed that chlorine<sup>5</sup> (50% of chlorine mixed with 50% distilled water) and Ivermectin<sup>5</sup> (5ml of Ivermectin (10%) mixed with 1 L of distilled water) were significantly effective in reducing biofouling and would not affect the health of seedlings.

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## APPENDIX 1

### Colonization of barnacles on *Avicennia marina* seedlings. (Experiment 1)

**1a)** 2 way ANOVA was used to test effect of time and section on abundance of barnacles.

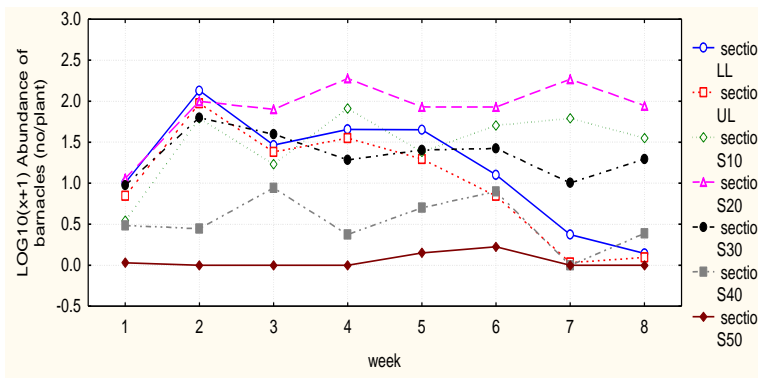
	Degr. of freedom	LOG10(x+1) no. barnacles (no/plant) SS	LOG10(x+1) no. barnacles (no/plant) MS	LOG10(x+1) no. barnacles (no/plant) F	LOG10(x+1) no. barnacles (no/plant) P
<b>Intercept</b>	1	744.11	744.11	1754.7	0.0000
<b>week</b>	7	44.06	6.29	14.8	0.0000
<b>section</b>	5	87.22	17.44	41.1	0.0000
<b>week*section</b>	35	56.53	1.62	3.8	0.0000

**1b)** Tukey HSD test; variable  $\log(x+1)$  abundance barnacles (time)

	week	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
<b>1</b>	1		0.00	0.00	0.00	0.00	0.00	0.00	0.00
<b>2</b>	2	0.00		0.26	1.00	0.16	0.28	0.37	0.01
<b>3</b>	3	0.00	0.26		0.46	1.00	1.00	1.00	0.91
<b>4</b>	4	0.00	1.00	0.46		0.31	0.49	0.60	0.03
<b>5</b>	5	0.00	0.16	1.00	0.31		1.00	1.00	0.97
<b>6</b>	6	0.00	0.28	1.00	0.49	1.00		1.00	0.89
<b>7</b>	7	0.00	0.37	1.00	0.60	1.00	1.00		0.81
<b>8</b>	8	0.00	0.01	0.91	0.03	0.97	0.89	0.81	

1c) Tukey HSD test; variable  $\log(x+1)$  abundance barnacles (section)

	section	{1}	{2}	{3}	{4}	{5}	{6}	{7}
1	LL		0.446	0.036	0.000	0.668	0.000	0.000
2	UL	0.446		0.000	0.000	0.006	0.000	0.000
3	S10	0.036	0.000		0.000	0.781	0.000	0.000
4	S20	0.000	0.000	0.000		0.000	0.000	0.000
5	S30	0.668	0.006	0.781	0.000		0.000	0.000
6	S40	0.000	0.000	0.000	0.000	0.000		0.000
7	S50	0.000	0.000	0.000	0.000	0.000	0.000	



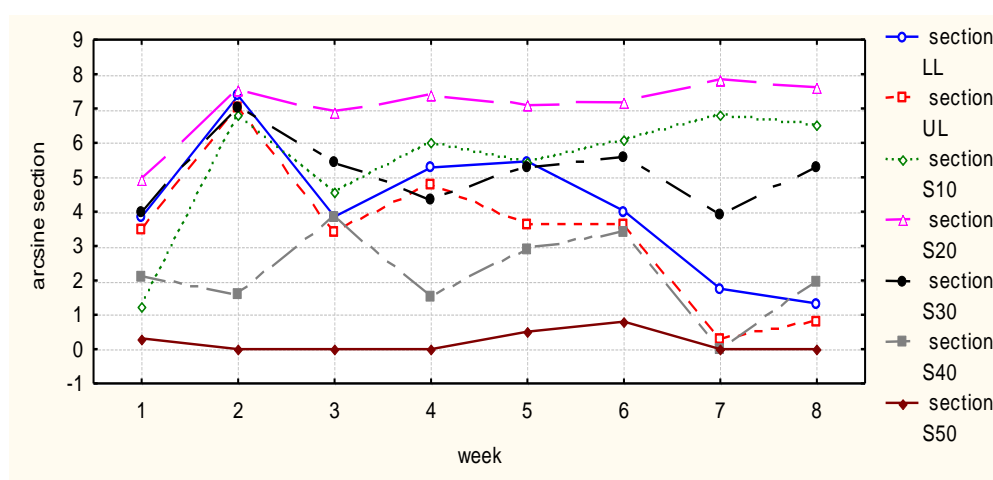
1d) Interaction effect of time and segment height on the abundance of barnacles (data was  $\log(x+1)$ )

1e) 2 way ANOVA was used to test effect of time and section on % cover of barnacles.

	arcsine section	arcsine section	arcsine section	arcsine section
	SS	MS	F	P
Intercept	8665	8665	1721	0.000
week	344	49	10	0.000
section	2474	412	82	0.000
week*section	727	17	3	0.000

**1f)** Tukey HSD test; variable arcsine transformation % cover (time)

week	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
1		0.000	0.001	0.000	0.000	0.000	0.000	0.000
2	0.000		0.000	0.003	0.000	0.001	0.003	0.001
3	0.001	0.000		0.284	1.000	0.621	0.270	0.517
4	0.000	0.003	0.284		0.559	0.999	1.000	1.000
5	0.000	0.000	1.000	0.559		0.876	0.542	0.802
6	0.000	0.001	0.621	0.999	0.876		0.999	1.000
7	0.000	0.003	0.270	1.000	0.542	0.999		1.000
8	0.000	0.001	0.517	1.000	0.802	1.000	1.000	



**1g)** Interaction effect of time and segment height on the % cover of barnacles (data was arcsine transformation))

## APPENDIX 2

### Colonization of barnacles on *Rhizophora apiculata* seedlings.

#### (Experiment 2)

**2a)** 2 way ANOVA was used to test effect of time and section on abundance of barnacles on *Rhizophora apiculata* seedlings

	Degr. Of freedom	LOG10 (x+1) SS	LOG10 (x+1) MS	LOG10 (x+1) F	LOG10 (x+1) P
<b>Intercept</b>	1	1.44	1.442	79.09	0.000
<b>week</b>	8	0.62	0.077	4.24	0.000
<b>section</b>	6	2.43	0.406	22.26	0.000
<b>week*section</b>	48	2.94	0.061	3.37	0.000

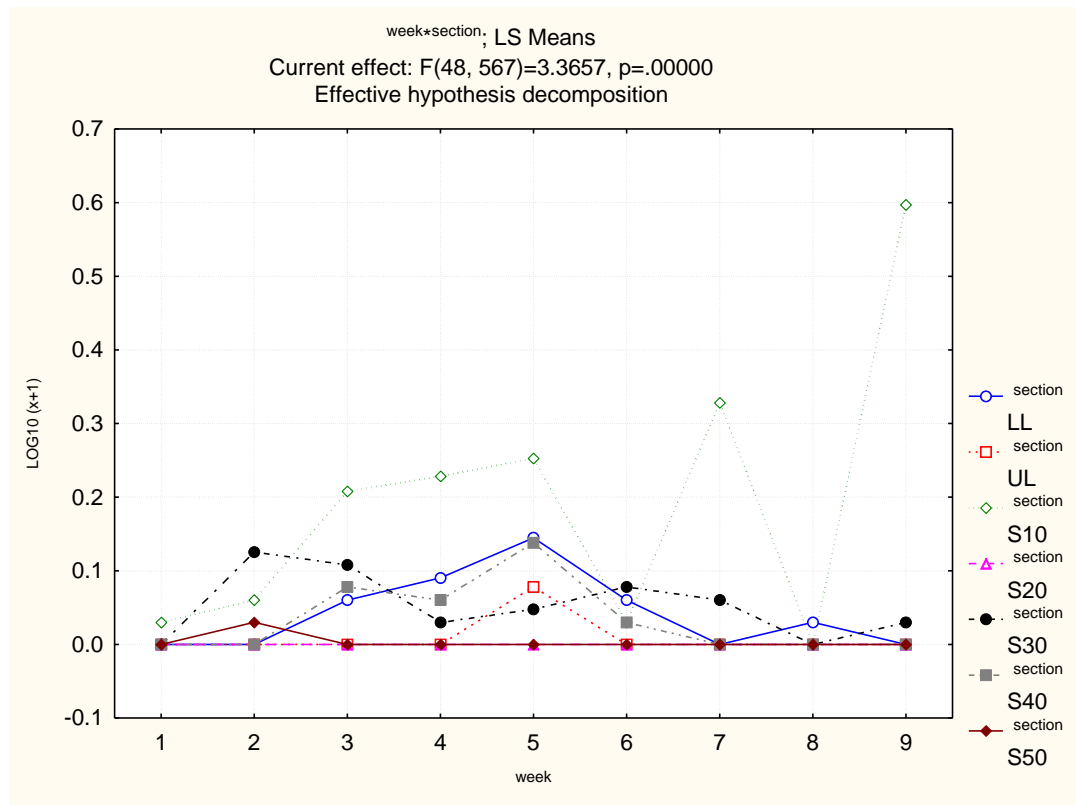
**2b)** Tukey HSD test; variable  $\log(x+1)$  abundance barnacles (time)

week	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}
<b>1</b>		0.964	0.165	0.300	0.003	0.981	0.377	1.000	0.006
<b>2</b>	0.964		0.861	0.955	0.119	1.000	0.977	0.964	0.197
<b>3</b>	0.165	0.861		1.000	0.933	0.805	1.000	0.165	0.977
<b>4</b>	0.300	0.955	1.000		0.817	0.926	1.000	0.300	0.911
<b>5</b>	0.003	0.119	0.933	0.817		0.089	0.744	0.003	1.000
<b>6</b>	0.981	1.000	0.805	0.926	0.089		0.959	0.981	0.153
<b>7</b>	0.377	0.977	1.000	1.000	0.744	0.959		0.377	0.859
<b>8</b>	1.000	0.964	0.165	0.300	0.003	0.981	0.377		0.006
<b>9</b>	0.006	0.197	0.977	0.911	1.000	0.153	0.859	0.006	



**2c) Tukey HSD test; variable  $\log(x+1)$  abundance barnacles (section)**

	section	{1}	{2}	{3}	{4}	{5}	{6}	{7}
1	LL		0.617	0.000	0.336	0.999	0.999	0.439
2	UL	0.617		0.000	1.000	0.286	0.870	1.000
3	S10	0.000	0.000		0.000	0.000	0.000	0.000
4	S20	0.336	1.000	0.000		0.112	0.623	1.000
5	S30	0.999	0.286	0.000	0.112		0.963	0.166
6	S40	0.999	0.870	0.000	0.623	0.963		0.730
7	S50	0.439	1.000	0.000	1.000	0.166	0.730	



**2d) Interaction effect of time and segment height on the abundance of barnacles (data was  $\log(x+1)$ )**

### APPENDIX 3

**3a)** Number of *Avicennia marina* leaves during barnacles infestation over a period of 8 weeks (Experiment 1)

1 way anova. To test effect of time on no. of leaves.

	SS	Degr. of	MS	F	p
<b>Intercept</b>	1575	1	1575	90.03	0.000
<b>week</b>	196	7	28	1.60	0.150

**3b)** Number of *Rhizophora apiculata* leaves during barnacles infestation over a period of 9 weeks (Experiment 2)

1 way anova. To test effect of time on no. of leaves.

	SS	Degr. of	MS	F	p
<b>Intercept</b>	1780	1	1780	716.8	0.000
<b>week</b>	157	8	20	7.9	0.000

**3c)** Tukey HSD test number of leaf (time)

	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}
<b>1</b>		0.552	0.005	0.007	0.027	0.001	0.003	0.031	0.000
<b>2</b>	0.552		0.464	0.597	0.856	0.181	0.438	0.854	0.000
<b>3</b>	0.005	0.464		1.000	0.999	1.000	1.000	1.000	0.047
<b>4</b>	0.007	0.597	1.000		1.000	0.999	1.000	1.000	0.018
<b>5</b>	0.027	0.856	0.999	1.000		0.967	0.999	1.000	0.005
<b>6</b>	0.001	0.181	1.000	0.999	0.967		1.000	0.978	0.120
<b>7</b>	0.003	0.438	1.000	1.000	0.999	1.000		1.000	0.024
<b>8</b>	0.031	0.854	1.000	1.000	1.000	0.978	1.000		0.008
<b>9</b>	0.000	0.000	0.047	0.018	0.005	0.120	0.024	0.008	

**3d) Shell diameter of barnacles on *Avicennia marina* seedlings. (Experiment 1)**

	Level of	Level of	N	Mean Size mm	Std.Dev Size mm
<b>Total</b>			356	2.05	1.39
<b>week</b>	1		48	0.78	0.27
<b>week</b>	2		51	1.22	0.30
<b>week</b>	3		53	1.22	0.48
<b>week</b>	4		43	1.64	0.87
<b>week</b>	5		50	2.08	0.99
<b>week</b>	6		48	2.63	1.11
<b>week</b>	7		29	4.48	0.75
<b>week</b>	8		34	3.96	1.26

**Effects of removal of biofouling organisms on survival of *Avicennia marina*, *Rhizophora mucronata* and *Rhizophora apiculata* seedlings (Experiment 3).**

**3e) Survival rates of *Avicennia marina* seedlings in different treatments.**

GENERAL Effect	Degr. of Freedom	sqrt Survival (week) SS	sqrt Survival (week) MS	sqrt Survival (week) F	sqrt Survival (week) p
<b>Intercept</b>	1	239.1252	239.1252	885.0239	0.000000
treatment	3	2.3902	0.7967	2.9488	0.053086

**3f) Mean number of *Rhizophora mucronata* leaves in different treatments.**

GENERAL Effect	Degr. of Freedom	(no/plant) SS	(no/plant) MS	(no/plant) F	(no/plant) p
<b>Intercept</b>	1	4508.544	4508.544	63.86686	0.000001
treatment	3	213.478	71.159	1.00802	0.418360

**3g) Mean number of *Rhizophora apiculata* leaves in different treatments.**

GENERAL Effect	(no/plant) SS	(no/plant) MS	(no/plant) F	(no/plant) p
<b>Intercept</b>	1720.026	1720.026	16.05909	0.002060
treatment	80.233	26.744	0.24970	0.859897

**3h) Survival rates of *Rhizophora apiculata* seedlings in different treatments.**

GENERAL Effect	Degr. of Freedom	Survival (%) SS	Survival (%) MS	Survival (%) F	Survival (%) p
Intercept	1	152100.0	152100.0	1521.000	0.000000
treatment	3	300.0	100.0	1.000	0.426221

**3i) Thickness of palisade layers (%)**

T-test for Dependent Samples (histology analysis) Marked differences are significant at  $p < .05$ .

	Mean	Std.Dv.	N	Diff.	Std.Dv. - Diff.	t	df	p
non fouled	56.80	1.643						
fouled	53.20	0.837	5	3.600	0.894	9.000	4	0.001

**3j) ANOVA: Number of abaxial hair in 500µm**

T-test for Dependent Samples (histology analysis) Marked differences are significant at  $p < .05$ .

	Mean	Std.Dv.	N	Diff.	Std.Dv. - Diff.	t	df	p
non fouled	5.600	0.548						
fouled	7.000	0.707	5	-1.40	1.140	-2.75	4	0.052

## APPENDIX 4

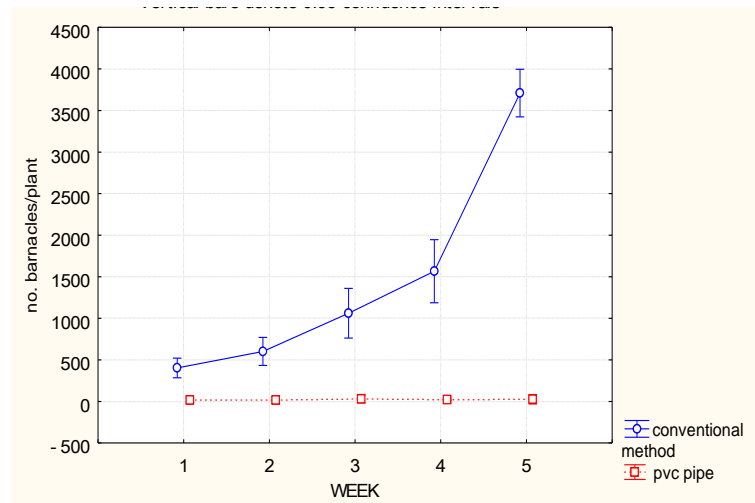
**Physical barrier to reduce biofouling (Experiment 4)**

**4a)** Repeated measure 2 factor ANOVA was used to analysis the effect of the weeks and treatments to the mean abundance of barnacles.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	16656710.5	1.0	16656710.5	831.9	0.0012
Error	40044.5	2.0	20022.2		
WEEK	10683588.5	4.0	2670897.1	741.9	0.0000
Error	28802.5	8.0	3600.3		
TREATMEN	15705014.5	1.0	15705014.5	1260.8	0.0008
Error	24912.5	2.0	12456.2		
WEEK*TREATMEN	10574271.1	4.0	2643567.8	859.4	0.0000
Error	24607.9	8.0	3076.0		

**4b)** Tukey HSD test shows the mean abundance of barnacles in conventional method (1) (1223.89 no/plant) was significant higher ( $p < 0.001$ ) compare physical barrier method (18 no/plant).

Cell No.	TREATMEN	{1}	{2}
1	1	1468.7	21.600
2	2	0.001128	



**4c)** The interaction effect of time and treatments on the abundance of barnacles

**4d)** Repeated measure 2 factor ANOVA was used to analysis the effect of the weeks and treatments on the abundance of leaf.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	841.0000	1	841.0000	10092.00	0.000099
Error	0.1667	2	0.0833		
WEEK	24.3333	5	4.8667	2.50	0.102403
Error	19.5000	10	1.9500		
TREATMEN	21.7778	1	21.7778	112.00	0.008811
Error	0.3889	2	0.1944		
WEEK*TREATMEN	6.2222	5	1.2444	0.85	0.544090
Error	14.6111	10	1.4611		

**4e)** Tukey HSD test shows the abundance of leaf in conventional method (1) (4.0566 no/plant) was significant lower ( $p < 0.001$ ) compare physical barrier method (2) (5.611 no/plant).

Cell No.	TREATMEN	{1}	{2}
1	1	4.0556	5.6111
2	2	0.009074	

## APPENDIX 5

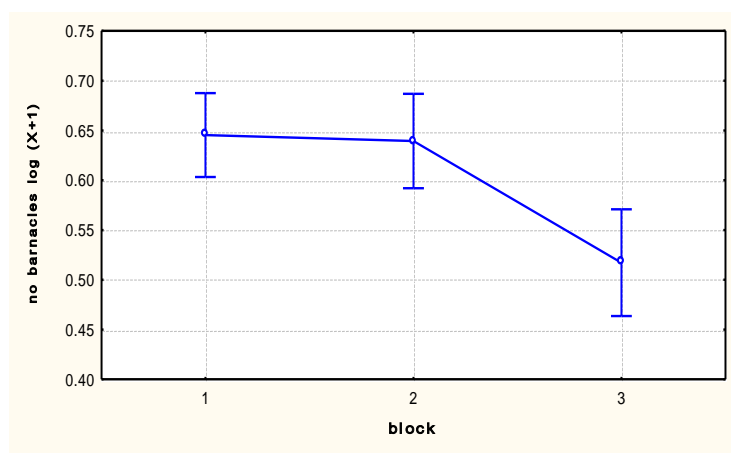
### Study on effectiveness of the elevated and short stake method of planting to reduce biofouling on *Avicennia marina* seedlings (Experiment 5)

**5a)** 2- way anova. Abundance of barnacles using different treatment, at different block over a period of 9 weeks. (Data was transform using  $\log(x+1)$ )

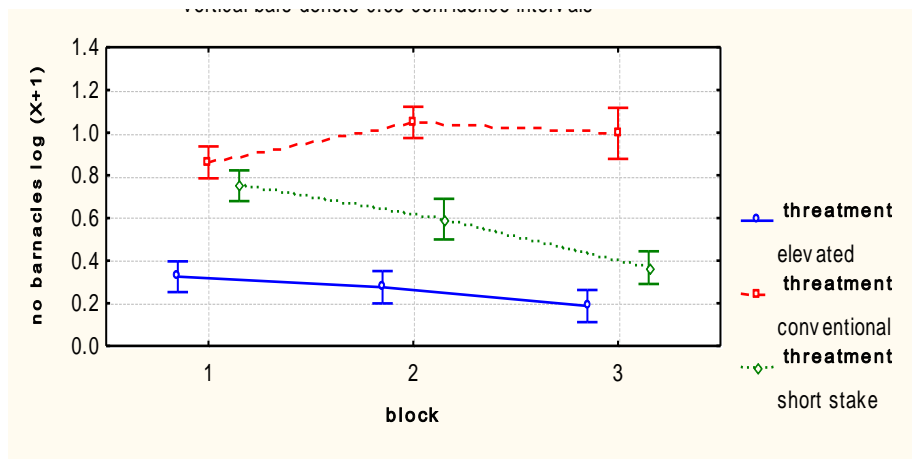
	SS	Degr. of	MS	F	p
block	1.5	2	0.7	8	0.000
treatment	39.2	2	19.6	207	0.000
block*treatment	3.9	4	1.0	10	0.000

**5b)** Tukey HSD. Number of barnacles using different treatment. (Data was transform using  $\log(x+1)$ )

	treatment	{1}	{2}	{3}
1	elevated		0.000	0.000
2	conventional	0.000		0.000
3	short stake	0.000	0.000	



**5c)** Effect of block on abundance of barnacles. (Data was transform using  $\log(x+1)$ )



5d) Interaction effect of block and treatments on abundance of barnacles. (Data was transform using  $\log(x+1)$ )

5e) Tukey HSD test. Effect of treatment on abundance of barnacles in the 1<sup>st</sup> week. (Data was transform using  $\log(x+1)$ )

Cell No.	threatment	{1}	{2}	{3}
1	elevated	.03010	.26251	.11379
2	conventional	0.001995		0.368941
3	short stake	0.368941	0.066009	

5f) Tukey HSD test. Effect of treatment on abundance of barnacles in 3<sup>rd</sup> week. (Data was transform using  $\log(x+1)$ )

Cell No.	threatment	{1}	{2}	{3}
1	conventional	.70779	.28732	.06608
2	short stake	0.000112	0.000112	0.000110
3	elevated	0.000110	0.008294	

5g) Tukey HSD test. Effect of treatment on abundance of barnacles in 6<sup>th</sup> week. (Data was transform using  $\log(x+1)$ )

Cell No.	threatment	{1}	{2}	{3}
1	conventional	1.4072	.81844	.31921
2	short stake	0.000113	0.000113	0.000115
3	elevated	0.000113	0.000115	

5h) Tukey HSD test. Effect of treatment on abundance of barnacles at the 9<sup>th</sup> week.  
(Data was transform using log(x+1))

Cell No.	threatment	{1}	{2}	{3}
		1.3300	.88932	.58389
1	conventional		0.006470	0.000118
2	short stake	0.006470		0.061611
3	elevated	0.000118	0.061611	

5i) one way Anova. Number of leaves in different treatment.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	3435.081	1	3435.081	452.90	0.0000
threatment	222.980	2	111.490	14.70	0.0000
Error	4065.406	536	7.585		

5j) Tukey HSD. Number of leaf using different treatment.

Cell No.	threatment	{1}	{2}	{3}
		3.3869	1.8500	2.3667
1	elevated		0.000022	0.000935
2	conventional	0.000022		0.195217
3	short stake	0.000935	0.195217	

5k) one way Anova. Number of leaves in different treatment in the 1<sup>st</sup> week.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	1251.031	1	1251.031	265.6245	0.000000
threatment	6.544	2	3.272	0.6947	0.502159
Error	381.492	81	4.710		

5l) one way Anova. Number of leaves in different treatment in the 3<sup>rd</sup> week.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	888.0951	1	888.0951	257.3487	0.000000
threatment	16.4001	2	8.2001	2.3762	0.099515
Error	272.6243	79	3.4509		



5m) one way Anova. Number of leaves in different treatment in the 6<sup>th</sup> week.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	121.2034	1	121.2034	32.06197	0.000000
threatment	31.8161	2	15.9080	4.20816	0.018745
Error	268.4002	71	3.7803		

5n) Tukey HSD. Number of leaf using different treatment in 6<sup>th</sup> week.

Cell No.	threatment	{1}	{2}	{3}
		.91304	.75000	2.1852
1	conventional		0.955612	0.061523
2	short stake	0.955612		0.027914
3	elevated	0.061523	0.027914	

5m) one way Anova. Number of leaves in different treatment in the 9<sup>th</sup> week.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	213.5382	1	213.5382	21.75788	0.000016
threatment	239.2422	2	119.6211	12.18846	0.000031
Error	647.7433	66	9.8143		

5o)Tukey HSD. Number of leaf using different treatment in 9<sup>th</sup> week.

Cell No.	threatment	{1}	{2}	{3}
		.27273	.75000	4.2963
1	conventional		0.874913	0.000197
2	short stake	0.874913		0.000912
3	elevated	0.000197	0.000912	

## APPENDIX 6

### Effects of biocidal chemicals on fouling organisms and *Avicennia marina* seedlings (Experiment 6)

6a)2 way- ANOVA was used to analysis the effect of time and treatments on abundance of barnacles.

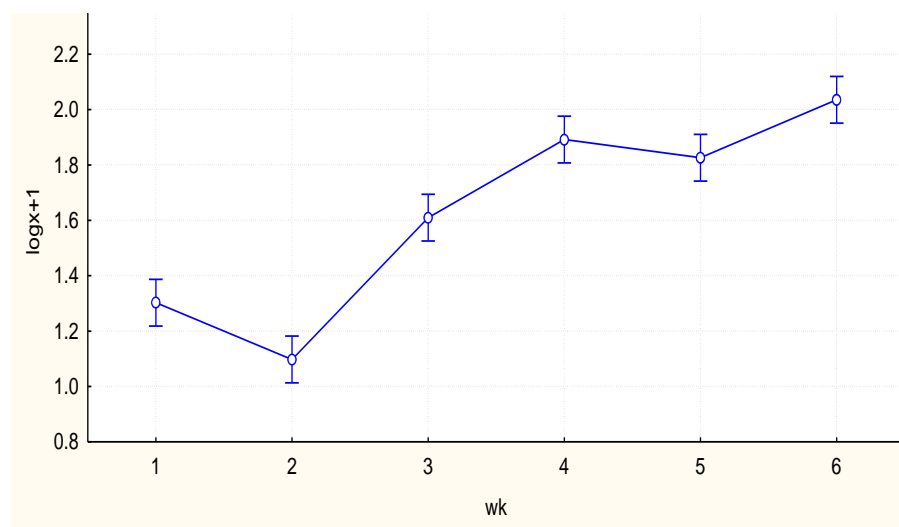
GENERAL Effect	Degr. of Freedom	total SS	total MS	total F	total p
Intercept	1	321963.2	321963.2	417.3872	0.000000
Week	5	115637.8	23127.6	29.9821	0.000000
treatment	4	29963.5	7490.9	9.7110	0.000004
Week*treatment	20	32647.8	1632.4	2.1162	0.013458

**6b)** Dunnett test was used to test whether any of the chemical treatments had an effect on biofouling by comparing each treatment mean against the control mean. (Experiment6)

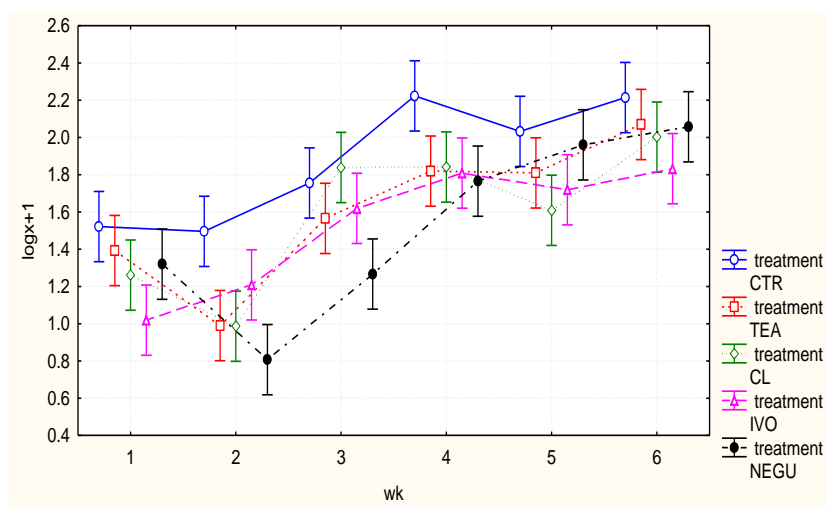
Dunnett test; variable logx+1 (experiment 6master)					
Probabilities for Post Hoc Tests (M<Control)					
Error: Between MS = .02668, df = 60.000					
Cell No.	treatment	{1}			
1	CTR	1.8741			
2	TEA	0.000027			
3	CL	0.000016			
4	IVO	0.000012			
5	NEGU	0.000012			

**6c)** Tukey HSD test. Effect of treatment and time (wk) to abundance of barnacles. The number of barnacles using Control was significantly different compare the other treatments.

Cell No.	treatment	{1}	{2}	{3}	{4}	{5}
1	CTR	95.333	53.222	52.222	43.056	55.222
2	TEA	0.000365		0.999972	0.806850	0.999555
3	CL	0.000289	0.999972		0.858808	0.997600
4	IVO	0.000136	0.806850	0.858808		0.683610
5	NEGU	0.000649	0.999555	0.997600	0.683610	



**6d)** Effect of time on abundance of barnacles.



**6e)** Interaction effect of the time and treatment to abundance of barnacles.

**6f)** Effect of time and treatment on number of leaves.

	SS	Degr. of	MS	F	p
<b>Intercept</b>	733.9	1	733.9	589.7	0.000
<b>wk</b>	64.7	5	12.9	10.4	0.000
<b>treatment</b>	2.5	4	0.6	0.5	0.733
<b>wk*treatment</b>	29.2	20	1.5	1.2	0.307

**6g)** Tukey HSD test. Effect of time on number of leaves.

week	{1}	{2}	{3}	{4}	{5}	{6}
<b>1</b>		1.000	0.963	0.000	0.002	0.002
<b>2</b>	1.000		0.963	0.000	0.002	0.002
<b>3</b>	0.963	0.963		0.002	0.021	0.021
<b>4</b>	0.000	0.000	0.002		0.963	0.963
<b>5</b>	0.002	0.002	0.021	0.963		1.000
<b>6</b>	0.002	0.002	0.021	0.963	1.000	

## APPENDIX 7

### Effects of different chemical concentrations on fouling of *Avicennia marina* seedlings (Experiment 7).

7a) the effects of chemicals of different concentration (code) and time (week) on the abundance of biofouling organisms. transform using (log x+1).

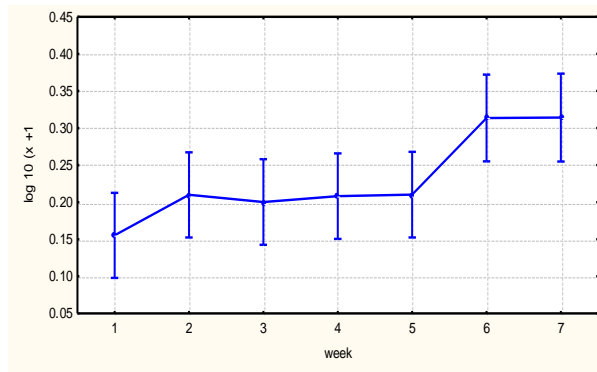
	SS	Degr. of	MS	F	p
<b>Intercept</b>	39.01	1	39.01	425.9	0.000
<b>week</b>	2.26	6	0.38	4.1	0.000
<b>CODE</b>	12.67	9	1.41	15.4	0.000
<b>week*CODE</b>	5.97	54	0.11	1.2	0.153

7b) Dunnett test was used to test whether any of the chemical treatments had an effect on biofouling by comparing each treatment mean against the control mean. (Experiment7)

		Dunnett test; variable log 10 (x +1 (experiment 7_CHEMICAL.sta)							
		Probabilities for Post Hoc Tests (M<Control)							
		Error: Between MS = .09158, df = 757.00							
		Include condition: $\sqrt{9}>5$							
Cell No.	CODE	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}
		.44007							
1	lvo1	0.000086							
2	lvo5	0.000022							
3	lvo10	0.000368							
4	CL1	0.019059							
5	CL5	0.000110							
6	CL10	0.000022							
7	NG1	0.000022							
8	NG5	0.000024							
9	NG10	0.000022							
10	CON								

7c) Tukey Test:effect of chemicals of different concentration (code) on abundance of barnacles, data was transform using logx+1.

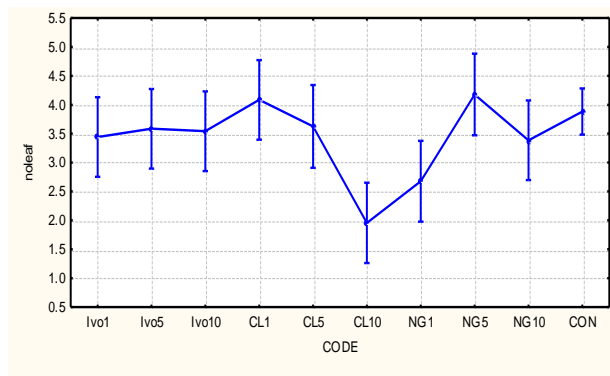
Cell No.	CODE	{1}	{2}	{3}	{4}	{5}	{6}	{7}	{8}	{9}	{10}
		.24575	.18314	.27401	.32373	.24964	.03926	.19018	.22408	.12399	.44007
1	lvo1		0.968737	0.999933	0.882742	1.000000	0.002348	0.986819	0.999994	0.337617	0.000154
2	lvo5	0.968737		0.750313	0.154631	0.959125	0.135394	1.000000	0.998714	0.978606	0.000012
3	lvo10	0.999933	0.750313		0.993787	0.999984	0.000214	0.835288	0.994092	0.096599	0.002883
4	CL1	0.882742	0.154631	0.993787		0.920963	0.000014	0.221362	0.650865	0.003753	0.142363
5	CL5	1.000000	0.959125	0.999984	0.920963		0.002350	0.981436	0.999978	0.319282	0.000433
6	CL10	0.002348	0.135394	0.000214	0.000014	0.002350		0.100380	0.013613	0.823127	0.000012
7	NG1	0.986819	1.000000	0.835288	0.221362	0.981436	0.100380		0.999737	0.957275	0.000013
8	NG5	0.999994	0.998714	0.994092	0.650865	0.999978	0.013613	0.999737		0.644768	0.000028
9	NG10	0.337617	0.978606	0.096599	0.003753	0.319282	0.823127	0.957275	0.644768		0.000012
10	CON	0.000154	0.000012	0.002883	0.142363	0.000433	0.000012	0.000013	0.000028	0.000012	



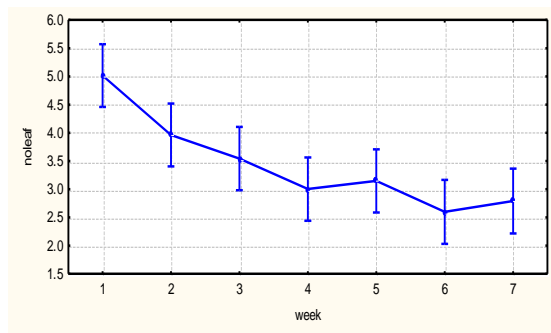
**7d)** effect of time on abundance of barnacles (data was transform using  $\log x + 1$ ).

**7e)** the effects of chemicals of different concentration (code) and time (week) on the number of leaves

	SS	Degr. of	MS	F	p
<b>Intercept</b>	8689	1	8689	1007	0.000
<b>week</b>	440	6	73	9	0.000
<b>CODE</b>	298	9	33	4	0.000
<b>week*CODE</b>	159	54	3	0	1.000



**7f)** effect of chemicals of different concentration (code) on number of leaves.



7g) effect of time on number of leaves

## APPENDIX 8

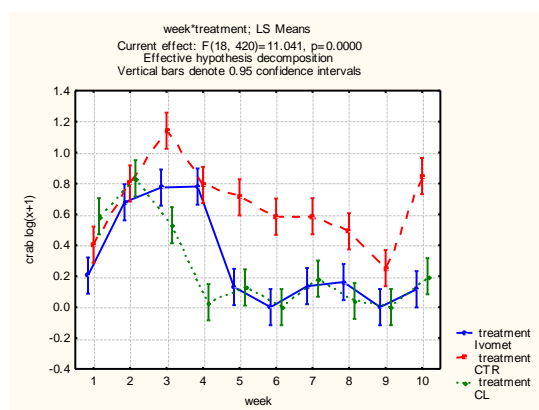
### Effects of biocidal chemicals on beach fauna at study site (Experiment 8)

8a).2-Way ANOVA .Effects of Week (1 to 10) and Treatment (Ivomect, CTR= Control, CL= Chlorine) to the density (no/m<sup>2</sup>) of **crab**.

GENERAL Effect	Degr. of Freedom	crab log(x+1) SS	crab log(x+1) MS	crab log(x+1) F	crab log(x+1) p
Intercept	1	73.49869	73.49869	1382.388	0.00
week	9	23.10475	2.56719	48.285	0.00
treatment	2	14.98602	7.49301	140.931	0.00
week*treatment	18	10.56658	0.58703	11.041	0.00

8b) (Tukey HSD Test) effect of treatment to **crab**

Cell No.	treatment	{1}	{2}	{3}
1	Ivomet	.29794	.66094	.25354
2	CTR	0.000022	0.000022	0.000022
3	CL	0.217773	0.000022	



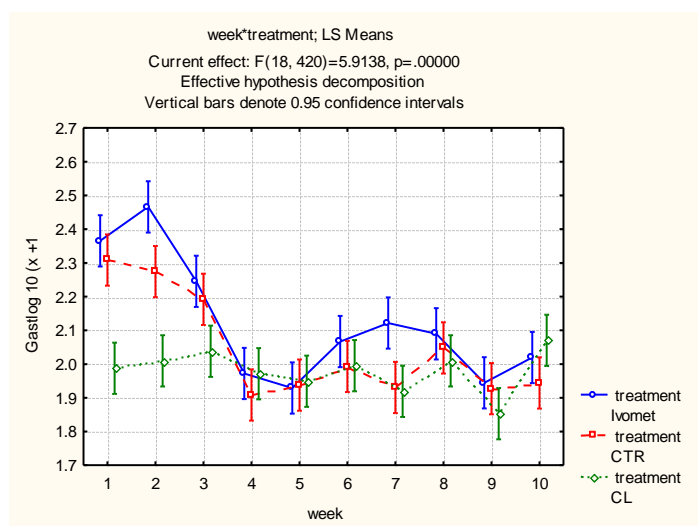
8c) Interaction effect of time and treatment on density of crabs. (Data was logx+1)

8d) 2-Way ANOVA .Effects of Week (1 to 10) and Treatment (Ivomect, CTR= Control, CL= Chlorine) to the density (no/m<sup>2</sup>) of **gastropods**.

GENERAL Effect	Degr. of Freedom	Gastlog 10 (x +1) SS	Gastlog 10 (x +1) MS	Gastlog 10 (x +1) F	Gastlog 10 (x +1) p
Intercept	1	1890.114	1890.114	84321.80	0.000000
week	9	5.814	0.646	28.82	0.000000
treatment	2	1.509	0.754	33.65	0.000000
week*treatment	18	2.386	0.133	5.91	0.000000
Error	420	9.415	0.022		
Total	449	19.123			

8e) (Tukey HSD Test) effect of treatment to **gastropods**.

Cell No.	treatment	{1} 2.1219	{2} 2.0463	{3} 1.9802
1	Ivomet		0.000056	0.000022
2	CTR	0.000056		0.000402
3	CL	0.000022	0.000402	



8f) Interaction effect of time and treatment on density of **gastropods**. (Data was logx+1)

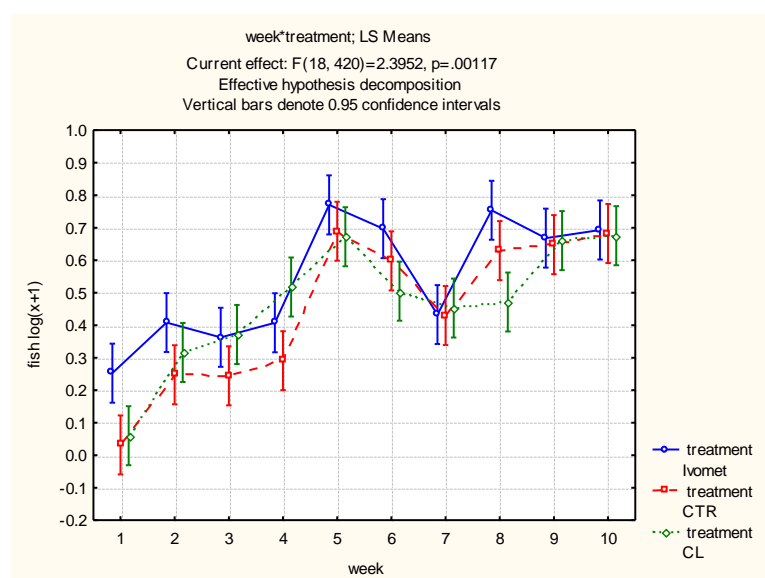
8g) 2-Way ANOVA and Post Hoc Test Results on the Effects of Week (1 to 10)

and Treatment (Ivomect, CTR= Control, CL= Chlorine) to the density (no/m<sup>2</sup>) of **fish**.

GENERAL Effect	Degr. of Freedom	fish log(x+1) SS	fish log(x+1) MS	fish log(x+1) F	fish log(x+1) p
Intercept	1	107.1568	107.1568	3347.661	0.000000
week	9	15.6600	1.7400	54.359	0.000000
treatment	2	0.7547	0.3774	11.789	0.000010
week*treatment	18	1.3800	0.0767	2.395	0.001174
Error	420	13.4440	0.0320		
Total	449	31.2387			

8h) (Tukey HSD Test) effect of treatment to **fish**

Cell No.	treatment	{1}	{2}	{3}
1	lvomet	.54459	.44908	.47028
2	CTR	0.000032		0.560085
3	CL	0.000951	0.560085	



8i) Interaction effect of time and treatment on density of **fish**. (Data was logx+1)